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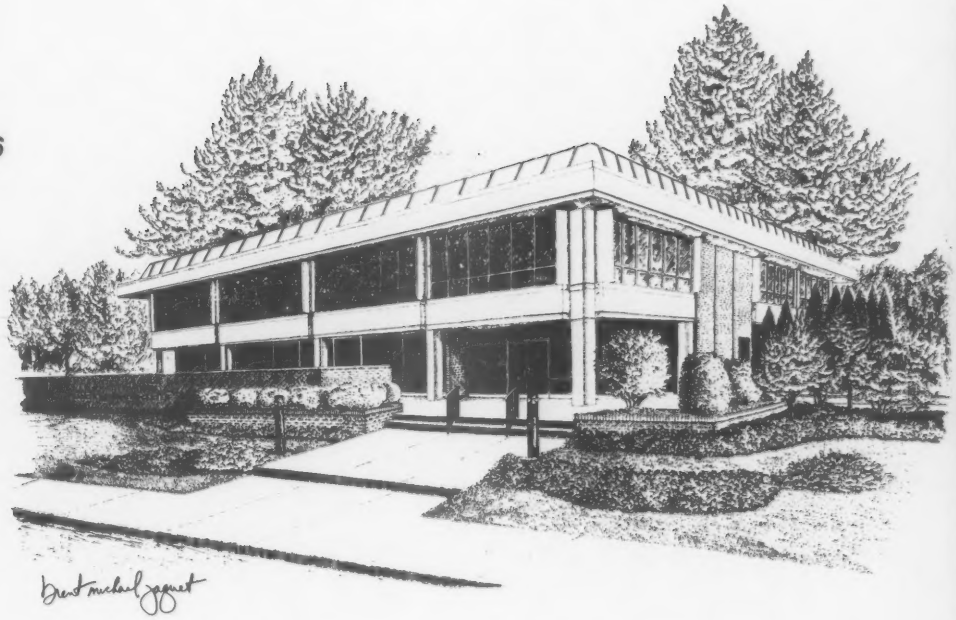

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Commentary on "Clinical Predictivity of Transplantable Tumor Systems in the Selection of New Drugs for Solid Tumors: Rationale for a Three-Stage Strategy"[1,2]

Daniel S. Martin,[3,4,*] Robert L. Stolfi,[4] and Robert C. Sawyer[4]

A recent report in this journal by Staquet et al (1), entitled as quoted above, analyzes the screening strategy used by the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (NCI), for the discovery of clinically effective drugs. In this analysis, the therapeutic results for 69 drugs that have been evaluated against both a NCI solid tumor animal panel of eight systems and a human solid tumor clinical panel of four systems are compared. On the basis of conclusions drawn from these comparisons, the cited report recommends certain changes in the national drug screening strategy. However, it appears likely that the conclusions reached through the elegant statistical study in this paper may be entirely incorrect, not because of inadequate statistical analysis, but because the data on chemotherapeutic sensitivity in the laboratory models simply are not comparable to available clinical data. Specifically, entirely different endpoints are utilized for determining therapeutic activity in the drug screening models and in the clinic. This difference was noted by the late Dr. Frank Schabel (2) as "the most important reason for the lack of clearly established positive correlation between claimed response to drug treatment of animal tumors and humans." Dr. Schabel then emphasized, "If we are to attempt to make meaningful and reliable comparisons between therapeutic activity of anticancer drugs in animals and man, we must use the same endpoints for measuring activity."

Staquet et al (1) defined their 69 drugs as clinically active if the drugs elicited tumor regression (≥ 50% in at least 10% of evaluable patients). In marked contrast, a very different therapeutic endpoint (inhibition of tumor growth) was the criterion of effectiveness employed to define the same antitumor agents as active in the animal tumor models. Thus, there is at least one very clear reason why there is a lack of correlation between the therapeutic results in the animal tumor models and those obtained in the human tumors. That is, differing methodologies and endpoints are employed to determine activity in the clinic versus the laboratory, and the laboratory methodology is the much more sensitive. Further, it is not valid to compare therapeutic activity against two different categories of tumor burden. Very extensive data have demonstrated that anticancer agents are much less effective against large numbers of cancer cells than on small numbers of the same tumor cells. In most of the animal tests, chemotherapy is initiated only 24 hours after transplantation on a small number of cells, usually only about 100,000. In the clinic tests, therapy is begun on a large (approximately 10^9 cells for a tumor as small as only 1 g) and well-established (likely present for ≥ 1 year) tumor.

With different endpoints being used to identify anticancer activity in humans and in animals, it is not surprising if the positive correlation between drug response of human cancer and animal models of human cancer is poor.

An appreciation of how disparate the measured tumor response to chemotherapy can be if different endpoints are compared can be discerned in the data in table 1. The chemotherapeutic results of screening ten drugs listed in table 1 against the same four tumors of the NCI tumor panel are obtained from two recent publications (1,3). Thus, both publications report the same data: ie, the same drugs on the same tumors and in the same laboratory hosts with the same methodologies conducted by the same NCI panel of investigators. It might be expected that this would result in identical correlations in activity. However, different criteria of positivity were employed by Staquet et al (1) than those utilized previously by Goldin et al (3) on the same screening data. Thus, the use of different endpoints resulted in varying correlations

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TABLE 1.—Two recent sets of NCI screening data demonstrating how disparate the measured tumor response to chemotherapy can be if different endpoints* are compared

Drug	Tumor pair CD (Staquet et al)†	Tumor pair CD (Goldin et al)‡	Tumor pair Co 38 (Staquet et al)†	Tumor pair Co 38 (Goldin et al)‡	Tumor pair B16 (Staquet et al)†	Tumor pair B16 (Goldin et al)‡	Tumor pair LL (Staquet et al)†	Tumor pair LL (Goldin et al)‡
Hexamethylmelamine	-	+	-	+	-	+	-	+
Chlorambucil	-	+	-	-	-	+	-	-
Methotrexate	-	+	-	-	-	-	-	+
Doxorubicin	+	+	-	-	+	+	-	+
Vincristine	-	+	-	+	+	+	-	-
Bleomycin	-	+	-	+	-	+	-	+
5-FU	+	+	+	+	-	+	-	+
Cyclophosphamide	+	+	-	+	+	+	+	+
Lomustine	+	+	-	+	+	+	+	+
Cisplatin	+	+	-	+	+	+	+	+
Correlations per tumor pair/No. of drugs compared	5/10 (50%)		4/10 (40%)		6/10 (60%)		5/10 (50%)	

* Activity criteria (Goldin et al): T/C (treated/control) expressed as % = ≥ 125% for B16 melanoma and ≥ 140% for LL (Lewis lung carcinoma); ≤ 42% for both Co 38 (Colon 38) and CD (CD_8F_1 mammary cancer). Activity criteria (Staquet et al): T/C % ≥ 150% for both B16 and LL; ≤ 10% for both CD and Co 38.

†*See* ref 1.

‡*See* ref 3.

that ranged from as low as 40% (the Co 38 tumor system) to no better than 60% (the B16 tumor system) (table 1). Against this example of disparity, it therefore is not surprising that there is a lack of correlation between drug treatment of animal tumors and human tumors, where not only the tumors but the staging, the methodologies, and, particularly, the endpoints are all different.

The lack of uniformity of criteria for activity measurement may seriously bias interpretations of data. In a forthcoming analysis (4) of essentially the same tumor panel screening data covered by Staquet et al (1), the NCI offered no conclusions regarding predictability because of the problem of comparing the different endpoints of tumor inhibition in the preclinical setting and response rate in clinical trials (4,5). Venditti (5) has noted that "expression of preclinical findings in terms of percentage responders against a specific type of cancer will permit more valid preclinical-clinical correlations because results of broad clinical trials are most often expressed in this way."

It is indeed possible to express preclinical activity as response rate. Laboratory tumors may be utilized as advanced-staged tumors and the therapeutic results can be reported, in addition to the degree of tumor growth inhibition relative to that of controls, as partial tumor regressions(s), complete tumor regressions(s), and increase in survival time and cure(s). Until the same endpoints for measuring therapeutic activity in the laboratory and the clinic are employed, the significance of comparisons such as those reported by Staquet et al (1) cannot be considered valid.

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Regional Intra-arterial Mitomycin C Infusion in Previously Treated Patients With Metastatic Colorectal Cancer and Concomitant Measurement of Serum Drug Level[1,2]

Mao H. Tseng,* John Luch, and A. Mittelman[3,4]

Fifty-seven patients with unresectable metastatic colorectal cancer, after failing all conventional chemotherapy, were treated with mitomycin C (MMC) regional intra-arterial infusion. The regional artery (eg, hypogastric, hepatic, etc) was approached percutaneously via the femoral artery and MMC at a dose of 20 mg/m² in 100 ml of 5% dextrose in water was infused for a 1-hour period; treatment was repeated every 6–8 weeks. Of 51 evaluable patients, five had objective response (three with pelvic tumor, one with liver and lung tumors, and one with liver tumors), 28 had stabilization of tumor, and 18 had no response. Median survival times for the responders, stabilized patients, and nonresponders were 46+, 39, and 22 weeks, respectively, with an overall survival of 32 weeks. The major side effect was necrotizing cellulitis occurring in the buttock following the pelvic infusion. Myelosuppression was manageable and other toxic effects were mild. Using the high-performance liquid chromatography method (total of 25 measurements), the average MMC levels in the peripheral circulation were 205, 62.4, and 16.0 ng/ml, respectively, immediately after injection and 1 and 2 hours following intra-arterial infusion. By 4 hours, no MMC could be detected in the peripheral circulation. [Cancer Treat Rep 68:1319–1324, 1984]

Unresectable metastatic or locally recurrent colorectal cancer remains one of the most refractory tumors to almost all treatments. Many drugs have been tested alone or in combination (1-3); none were demonstrated to be highly effective. 5-FU remains the most useful agent but only produces a partial response in 20% of the patients (4) and does not increase patient survival (5,6). To enhance 5-FU antitumor activity, regional intra-arterial (ia) infusion has been developed. However, complicated infusional procedures plus drug side effects may have outweighed the benefit of ia therapy (7-10). Enzymatic manipulations (11,12) of 5-FU to potentiate intracellular biochemical pathways resulted in a failure to increase anticancer potential combined with significant host toxicity. When patients failed to respond to an initial chemotherapy trial, subsequent conventional treatment usually proved to be ineffective (13,14).

One possible explanation for this poor therapeutic result was the inability of current modalities to attack the hypoxic cancer cells present in solid tumors (15). Mitomycin C (MMC), discovered in 1956 by Hata et al (16), had demonstrated a broad spectrum of anticancer potential (17,19). However, cumulative myelosuppression, which occurred when MMC was given iv, limited usage of this drug, mainly to small dosages or as a component of combination therapy. Recent studies suggested that MMC is not only able to attack well-oxygenated cancer cells (proliferative), but also showed increased cytotoxic activity against the hypoxic cell (nonproliferative). This increased cytotoxicity disappeared when cells became well-oxygenated (15).

To further evaluate the antineoplastic potential of MMC, the drug was given through a regional artery in patients with advanced localized colorectal cancer. In addition, to increase the understanding of its pharmacokinetics in humans, determination of MMC level in the blood following infusion was conducted.

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[2]Supported in part by Institution grant IN-54-426 from the American Cancer Society.
[3]Department of Surgical Oncology, Roswell Park Memorial Institute, Buffalo, NY.
[4]We thank Dr. H. Douglass, Jr, for reviewing this paper; Drs. H. Park, J. Park, Patel, Ghoorah, and Barua for technical assistance; Robert Moore for analyzing the data; and Sandra Alderman for data collection.
* *Reprint requests to:* Mao H. Tseng, MD, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

MATERIALS AND METHODS

From March 1980 to November 1983, 57 patients with histologically proven, unresectable, metastatic colorectal adenocarcinoma, previously unresponsive to conventional chemotherapy, were treated with ia MMC infusion. Of the 57 patients entered, six have been lost to follow-up. Of 51 evaluable patients, 29 were male, with a median age of 60 years (range, 40–74), and 22 were female, with a median age of 48 years (range, 28–65). Arteries selected for infusion depend on the location of the tumor (table 1). Fourteen patients had tumor confined to the pelvis and 14 patients had metastases only in the liver. In the remaining 23 patients, tumor involved two or more organs.

On the first infusion, a regional arteriogram was performed to identify the blood vessels supplying the cancer. If no obvious tumor vessel was seen, the closest major arterial supply to the tumor was infused. At subsequent treatments, arteriogram was not needed. The infusion catheter was inserted percutaneously through the femoral artery to the designated artery and 20 mg/m^2 of MMC in 100 ml of 5% dextrose in water was infused for 1 hour. When two organs were involved, infusion went either to the symptomatic site or one-half of the MMC was given to one site and the remaining one-half was then infused through a second vessel after repositioning the catheter. The catheter was removed immediately after infusion and the patient was discharged on the following day. Weekly cbcs were done thereafter. The tumor status was evaluated with chest x-ray, and liver, bone, and CAT scans accordingly every 6–8 weeks before treatment was repeated.

The protocol required pretreatment wbc counts of $> 4000/mm^3$, platelet counts of $> 100,000/mm^3$, hemoglobin of > 10 g/dl, and hematocrit of $> 30\%$. Anemia was corrected by transfusion. Patients with renal or hepatic failure did not qualify for this treatment. Treatment was discontinued when tumor showed progression, which was defined as increasing the measurement of tumor by $\geq 25\%$ as determined by the product of two perpendicular diameters, or when severe host toxicity occurred. Treatment was continued as long as tumor remained stable or showed partial response ($\geq 50\%$ tumor reduction by the two perpendicular measurements).

TABLE 1.—Location of tumor and arteries for infusion in 51 patients with colorectal cancer

Site of tumor	No. of patients	Artery for infusion
Pelvis	14	Hypogastric or superior gluteal
Liver	14	Hepatic or celiac
Lung and liver	6	Hepatic
Liver, lung, and pelvis	2	Hepatic and hypogastric
Liver and pelvis	2	Hepatic and hypogastric
Liver and bone	1	Hepatic
Pelvis and bone	4	Hypogastric
Lung and pelvis	5	Hypogastric
Mesentery of the colon and retroperitoneum	3	Jejunal branch of superior mesenteric artery

With each infusion, peripheral blood samples (3 ml each) were taken before and immediately after and 1, 2, 4, 6, 8, 10, and 24 hours after infusion for the measurement of MMC level. The serum was prepared and monitored with high-performance liquid chromatography (HPLC) effluent at 365 nm by the method of Kono et al (29).

RESULTS

Five patients achieved partial response, which included three of 25 patients receiving hypogastric infusion and two of 23 patients receiving hepatic infusion for their pelvic and liver metastases, respectively (table 2). In 13 patients with pelvic tumors and in 15 with liver metastasis, the infusional therapy was able to stabilize their disease for > 2 months. In 18 patients, the treatment was not effective; these patients included five with pelvic tumors, six with liver tumors, four with pelvic plus bony metastasis, and three with retroperitoneal tumors. Two of the three responders with pelvic tumors had pelvic pain. Following the first hypogastric infusion, pain was reduced significantly, beginning shortly after infusion and lasting 4–5 weeks. The pain relief was reinduced on subsequent treatments at 6-week intervals. This kind of pelvic pain relief was also observed in eight patients with tumor-stabilization and in four whose tumor did not respond. In one patient who had liver and lung metastases, the infused liver and the multiple lung metastasis regressed significantly. This response was accompanied by a fall to normal of the elevated serum alkaline phosphatase (it remained normal during the entire period of response). One patient with liver metastasis has responded continuously and is still alive and active at 15 months since the beginning of the infusion.

Responders received an average of four infusions and a total of 164 mg of MMC. The median survival time from the day of infusion was 46 weeks. Among the 18 nonresponders, temporary, less than partial, objective, and subjective response were noted, lasting < 2 months. For nonresponders, median survival was only 22 weeks, and the average total MMC received per patient was 63 mg in 1–2-hour infusions. The 28 patients with stable disease had a median survival of 39 weeks, receiving an average total MMC dose of 81 mg in three infusions.

TABLE 2.—Characteristics of 5 patients with partial response

Patient No.	Age (yrs)/sex	Location of tumor ;	Performance score*	Artery for infusion	Total courses of infusion/total dose (mg) of MMC	Evaluation of metastasis
1	74/M	Liver and lung	0	Hepatic	3/110	Chest x-ray and liver scan
2	59/M	Pelvis and lung	1	Hypogastric	6/191	Pelvic scan
3	68/M	Pelvis	1	Hypogastric	3/106	Pelvic scan
4	66/M	Pelvis	2	Hypogastric	4/152	Palpation, 28 × 20 cm (8/80) to 20 × 10 cm
5	58/M	Liver	0	Hepatic	7/260	Liver scan

*Eastern Cooperative Oncology Group criteria: 0 = asymptomatic; 1 = symptoms but ambulatory; and 2 = recumbent < 25% of day.

The myelosuppression observed in 123 consecutive ia infusions showed that nearly 50% of patients had none to minimal toxicity. Toxicity was reversible except in one patient (table 3). The major side effect encountered was necrotizing cellulitis (fig 1) which occurred in three patients following MMC infusion through the hypogastric or superior gluteal arteries. Among these three patients, one had partial response which lasted 15 months and one had tumor stabilization for 8 months. These two patients also experienced significant relief of their pelvic pain after each infusion; the third patient was asymptomatic and the infusion therapy stabilized the tumor for 14 months. The cellulitis was present initially in the buttock, and the surrounding skin showed brownish pigmentation. The reaction was accompanied by a moderate degree of burning pain; usually the skin change became stable and gradually the pigmentation and the buttock pain faded. In these patients skin ulceration developed, which required debridement. With conventional wound care, these wounds all healed eventually and without further complication. This skin change was an indication for termination of the infusion. If no tumor progression occurred, MMC was subsequently given by iv infusion. The other toxic effects were mild degrees of nausea and vomiting. These were usually transient and occurred only occasionally. There was no renal or pulmonary toxicity observed.

Determination of the serum MMC following infusion with HPLC showed the concentration of MMC in the peripheral blood immediately after infusion and at intervals of 1 and 2 hours following ia MMC infusion to be 205.12 ± 28.99, 62.44 ± 13.65, and 16.08 ± 6.36 ng/ml, respectively. MMC level declined rapidly and could not be detected 4 hours after infusion (table 4).

DISCUSSION

Regional ia infusion for localized unresectable tumor is theoretically more attractive than chemotherapy given iv (20,21). However, in colorectal cancer metastatic to the liver, the results of hepatic infusion have been inconsistent. No series presents a survival advantage from ia infusion compared to iv chemotherapy (9). In addition, prolonged catheterization and hospitalization make the infusional therapy unattractive and very discouraging to patients. Using the recently developed implantable pump system, the ia chemotherapy can be given in a much easier fashion. In a reported series of 13 patients who received hepatic infusion, 11 achieved partial response (22). The results were good; however, it will be more convincing if similar antitumor activity can be generated from a larger and randomized study. In addition, the requirement of an initial surgical procedure will limit this therapy to patients with good surgical risk. For patients who have failed all conventional chemotherapy or radiotherapy, our intermittent one-shot infusion appears to be more convenient and suitable to the patient.

MMC, an alkylating agent, is capable of attacking

TABLE 3.—Bone marrow toxic effects*

Grade	Blood cell counts (cells/mm^3)		No. of infusions
	Wbc	Platelet	
0 (normal)	> 4000	> 100,000	43
I	3000-3999	75,000-99,999	22
II	2000-2999	50,000-74,999	38
III	1000-1999	25,000-49,999	16
IV	< 1000	< 25,000	4
Total			123

*All bone marrow toxicity was reversible except 1 case.

FIGURE 1.—Necrotizing cellulitis, with brownish pigmentation of surrounding skin.

cancer cells in all phases. The best evidence is its demonstrated ability to achieve some objective response, even in previously treated patients (23). Nevertheless, MMC is usually given in small doses or as a part of combination therapy because of its severe and cumulative bone marrow toxicity, a frequent complication after systemic MMC therapy. The poor results of a MMC-containing regimen given to patients who failed to respond to 5-FU indicated the lack of antitumor activity of MMC when given iv. In the series of MMC combined with cyclophosphamide and methotrexate, only one of 14 patients had a partial response that lasted > 2 months, with a median survival of 4.3 months (24). In another series, a combination of MMC plus doxorubicin and 5-FU showed none of the 21 patients responded (25). This could be due partly to the small dose (10 mg/m^2) of MMC given in those series. As shown in this series, MMC given in higher doses (20 mg/m^2) ia shows not only a much better clinical response accompanied by longer survival but also produces very manageable myelosuppression. Such tolerable toxicity could be due to the fact that the strict requirement of a normal blood cell count (eg, wbc count > 4000/mm^3, platelet count > 100,000/mm^3, hemoglobin > 10 mg/dl, and hematocrit > 30%) prior to each infusion required even a longer interval between treatment.

Metastatic colorectal cancer, whether in the liver or in the pelvis, is essentially a vascular-deficient tumor, judging from the arteriogram. Therefore, the amount of tumor that may become hypoxic is relatively higher than that of tumors that are more vascular. The lack of vasculature and the depletion of tissue oxygenation increases the proportion of hypoxic cells that have been shown to be resistant to radiotherapy and chemotherapy (26,27). Recent experimental work (28) showed that MMC, under hypoxic condition, underwent a bioreductive reaction and exhibited preferential cytotoxicity to hypoxic cells. This kind of activity disappeared when

TABLE 4.—MMC serum level following ia or iv infusion*

	Time (hrs)†			
	0	1	2	4
Ia (25 infusions)	205.12 ± 28.99‡	62.44 ± 13.65	16.08 ± 6.36	0 ± 0
Iv (22 infusions)	134.2 ± 29.99	20.08 ± 4.84	4.76 ± 2.16	0.6 ± 0.45

*Dose of MMC given/infusion: 20 mg/m^2 ia and 15 mg/m^2 iv.
†Time blood sample taken following the end of infusion.
‡Mean ± SE (ng/ml).

the environment changed from hypoxic to nonhypoxic conditions. To assure such activity, one has to make sure the MMC can be delivered to the solid tumor; the regional artery which supplies the tumor area provides the most accessible route for such purpose. There was no correlation between the relief of pain and the tumor response, since all symptomatic patients, including the nonresponders, have experienced relief of their pain; however, the immediate reduction or stoppage of the narcotic medication following the regional infusion made this therapy highly acceptable, even in the absence of objective response.

The few data available in the area of pharmacokinetics of MMC in humans were basically due to the lack of a proper, sensitive biochemical assay to measure MMC. Recent advances of HPLC technique have enhanced our ability to detect even trace amounts of the drug in humans (29).

In 25 consecutive analyses of ia infusions (20 mg/m^2), the MMC level at 0, 1, and 2 hours was found to be higher than in a group of patients who received a smaller but therapeutic dose of MMC (15 mg/m^2) iv (table 4). Following the ia infusion, good therapeutic concentration of MMC in the peripheral circulation remained for at least 2 hours. Possibly this systemic MMC level had antitumor activity against the distant lung metastasis even after hepatic infusion. The higher serum MMC level following ia infusion was possibly due to the larger MMC dose given per each infusion. An average of 33.4 mg of MMC was given by ia infusion versus 24.5 mg of MMC given by iv infusion. In addition, the high plasma level of MMC suggested the infused tissue cleared the drug poorly.

The tremendous relief of pelvic pain with the pelvic infusion, even in the presence of other metastasis, suggests a good palliative role for this therapy. However, experience shown in this series indicated that when tumor involved the retroperitoneum, mesentery, or bone, the regional MMC infusion was ineffective because there was no accessible artery for infusion. The antitumor activity after hepatic infusion of 23 patients with liver metastasis alone or combined with lung metastases was comparable to other infusion series (8,30).

Despite extensive clinical trials, chemotherapy for previously treated patients with metastatic, unresectable colorectal cancer remains unimproved (31-35). All modalities available at this stage can only offer, at the most, slight palliation of symptoms. Percutaneous regional MMC ia infusion given intermittently was an effective way of palliation of pelvic pain and improved the quality of these patients' lives.

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Chemotherapy for Metastatic Non-Small Cell Bronchogenic Carcinoma: Cyclophosphamide, Doxorubicin, and Etoposide Versus Mitomycin and Vinblastine (EST 2575, Generation IV)[1,2]

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Between March and December 1979, the Eastern Cooperative Oncology Group (ECOG) compared the CAVP16 (cyclophosphamide, doxorubicin, and etoposide) and MV (mitomycin and vinblastine) regimens in 200 patients with metastatic non-small cell bronchogenic carcinoma. Most patients were ambulatory (ECOG performance status 0, 1; 75%) and had not received prior radiation therapy (76%). The CAVP16 regimen resulted in one complete and 13 partial responses (14%) and the mitomycin and vinblastine regimen resulted in two complete and 11 partial responses (13%). These results did not differ significantly ($P = 0.45$) nor did the results for median survival, 18 weeks for each regimen. Good performance status and prior surgical resection were positive predictors of improved survival. Toxicity was significant with eight drug-related deaths, six due to leukopenia and infection and one each due to renal failure and interstitial pneumonitis. CAVP16 resulted in significantly more serious and life-threatening toxicity ($P < 0.0001$). Neither regimen is recommended for further study. [Cancer Treat Rep 68:1325–1329, 1984]

The search remains active for an effective chemotherapeutic regimen for metastatic non-small cell bronchogenic carcinoma. Despite numerous reports of efficacious combinations from single institutions (1–5), the definitive acceptance of such a regimen requires confirmation in a cooperative group trial (6). EST 2575 was a prospective, randomized clinical trial of the Eastern Cooperative Oncology Group (ECOG) that served to screen for activity of new regimens and to evaluate groupwide efficacy of treatments reported to show activity. The first three generations of this trial have been reported previously and served to confirm modest activity for three regimens and to offer negative results for a series of single agents and combinations (7–9). Generation IV of this study accrued 219 patients between March and December 1979. It compared the responses and survival of patients treated with either a combination of cyclophosphamide, doxorubicin, and etoposide (CAVP16) (10) or the combination of mitomycin and vinblastine (MV) (11).

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TABLE 1.—Dose schedules for the CAVP16 and MV regimens

Regimen	Compound	Dose (mg/m^2)	Schedule and route
CAVP16	Cyclophosphamide	400	Days 1 and 8 iv, q 28 days
	Doxorubicin	30	Days 1 and 8 iv, q 28 days
	Etoposide	100	Days 1 and 8 iv, q 28 days
MV	Mitomycin	10	Day 1 iv, q 21 days
	Vinblastine	6	Day 1 iv, q 21 days

PATIENTS, MATERIALS, AND METHODS

Patients

For entry in this trial, patients were required to have histologically confirmed, metastatic, non-small cell bronchogenic carcinoma. Hilar or mediastinal node involvement as the sole site of metastases was not acceptable for study whether or not the patient was technically resectable. Patients with squamous, large cell anaplastic carcinoma and adenocarcinoma (including bronchoalveolar carcinoma) were accepted, while those with small cell anaplastic carcinoma were excluded. Patients were required to be at least partially ambulatory (ECOG performance status 0, 1, or 2) and to have had no prior chemotherapy, no prior radiation to the area of measurable disease, no CNS metastases, and no symptomatic cardiac disease (defined as congestive heart failure, myocardial infarction within 3 months, unstable angina, unstable arrhythmia, or uncontrolled hypertension). Acceptable baseline organ function was defined as creatinine < 1.5 mg/dl, bilirubin < 2.0 mg/dl, wbc count $\geq 4000/mm^3$, and platelet count $\geq 100{,}000/mm^3$. As described earlier (9), this defines a more homogeneous group of patients with metastatic lung cancer who have a reasonably good performance status. All patients gave informed consent to participate in this trial.

Study Design and Treatments

Patients were stratified by histologic cell type and initial performance status (0 or 1 vs 2). The doses and schedules for the two treatment regimens are shown in table 1. Doses were modified on the basis of wbc and platelet counts on the day of treatment and at the estimated midcycle nadir as described in detail elsewhere (12). Dose adjustments were also permitted for hepatic or renal dysfunction, severe nausea and vomiting, severe mucositis or cystitis, and significant neurotoxicity. Details of ECOG response and toxicity criteria have been recently published (13). In summary, toxicity is graded on a six-point scale (0 = none to 5 = fatal) and is organ- or site-specific. Response is defined strictly as complete or partial (> 50%). Minimal responses (< 50%) and regressions are considered stable disease.

RESULTS

Patient Characteristics

Of 219 patients entered between March and December 1979, 200 were eligible for analysis, 99 on the CAVP16 arm and 101 on the MV arm. Of the 19 unevaluable patients, five were cancelled prior to therapy and 14 were ineligible based on wrong histology (three patients), absence of metastases (one), brain metastases (four), second malignancy (one), prior radiation to measurable disease (two), absence of any evaluable disease (one), and abnormal baseline organ function (two). Table 2 shows prog-

TABLE 2.—Distribution of eligible patients by treatment and selected prognostic factors

Prognostic factor	No. of patients treated with— CAVP16 ($n = 99$)	MV ($n = 101$)
Cell type		
Squamous cell carcinoma	35	35
Adenocarcinoma	46	46
Large cell anaplastic carcinoma	16	19
Mixed adenosquamous cell carcinoma	2	1
Initial performance status		
0	23	15
1	52	60
2	24	26
Sex		
Male	67	68
Female	32	33
Prior weight loss		
None	40	48
< 5%	24	20
5%–10%	16	8
> 10%	16	22
Unknown	3	3
Prior radiotherapy		
Yes	73	79
No	26	22
Prior surgery		
Yes	60	44
No	39	57
Age (yrs)		
< 50	17	12
50–59	36	40
60–69	33	31
≥ 70	12	11
Unknown	1	7

nostic factors found to be of importance in prior generations of EST 2575 (7-9) and shows their distribution by treatment. Only prior surgery was not balanced and this was noted and adjusted for in the response and survival analysis.

Response

All patients but one were included in the response analysis. No follow-up was received on this patient other than that he was known to be alive 393 days following randomization. The overall response rate for CAVP16 (14%, one complete response) was not significantly different from that for MV (13%, two complete responses) (P = 0.45, one-tailed Fisher exact test) (14). The 95% confidence interval (Gaussian approximation) for CAVP16 is 9%-23% and for MV is 8%-21%. The median time to response for the 27 responders was 40 days. The median duration of response was 87 days. The median duration of response was longer for the patients receiving CAVP16 than for those receiving MV (14.6 vs 8.1 weeks, P = 0.03; two-sample log-rank test) and for patients with adenocarcinoma (P = 0.04; three-sample log-rank test). Table 3 shows that response did not vary by age, sex, cell type, performance status, prior radiotherapy, prior surgery, or prior weight loss.

Survival

All 200 patients were included in the survival analysis. Six of the 200 patients were still alive at the final analysis in August 1981 (censored failure time). The overall median survival was 18 weeks. The longest survival times were 97 weeks on CAVP16 and 93 weeks on MV. Table 3 analyzes survival by the same criteria used for response. To avoid potential biases caused by imbalance of prognostic factors in subgroups of patients, an analysis of the survival data was conducted using the proportional hazards model of Cox (15-17). For the variables which are not dichotomous (performance status and prior weight loss), levels were assigned consecutive integer scores and a linear effect was investigated. When each factor was treated separately, the following were correlated with poor survival: poor performance status (P = 0.001), no prior surgery (P = 0.02), and prior weight loss (P = 0.02). Neither treatment nor histologic subtype correlated with survival. Other insignificant factors included age, sex, and prior radiotherapy. Responders to

TABLE 3.—Objective tumor response and survival by selected prognostic factors

Prognostic factor	No. of patients treated	No. of responders	% response rate (complete + partial)	Median survival (wks)
Cell type				
Squamous cell carcinoma	70	10	14	14.6
Adenocarcinoma	92	12	13	20.6
Large cell carcinoma	35	5	14	16.2
Performance status				
0	38	3	8	24.3
1	112	21	19	19.4
2	50	3	6	10.9
Sex				
Male	135	20	15	17.7
Female	65	7	11	19.1
Prior weight loss*				
None	88	13	15	24.3
< 5%	44	4	9	14.6
5%-10%	24	3	12	17.4
> 10%	38	6	16	12.0
Prior radiotherapy				
Yes	48	8	17	16.3
No	152	19	13	19.1
Prior surgery				
Yes	104	16	15	21.4
No	96	11	12	16.7
Age (yrs)*				
< 50	29	2	7	18.6
50-59	76	12	16	17.1
60-69	64	8	13	20.3
⩾ 70	23	4	17	16.4

*Patients with unknown level of factor are omitted.

TABLE 4.—Analysis of toxicity by treatment

Type of toxicity	No. of patients with severe, life-threatening, or fatal toxic reactions*	
	CAVP16 (n = 99)	MV (n = 101)
Hematologic	41 (4)	14 (1)
Infection	6 (2)	1
Vomiting	18	3
Diarrhea	0	2
Gastrointestinal (other than vomiting)	1	0
Respiratory	1	1 (1)
Neurologic	0	2
Cardiac	0	0
Skin, mucosa	0	1
Genitourinary	0	1 (1)
Hepatic	0	1

*Nos. in parentheses = No. of deaths attributed to therapy.

either regimen lived significantly longer than nonresponders, 35.3 versus 16.7 weeks (P = 0.003, log-rank test).

Toxicity

Of the 200 patients treated, all were available for at least partial analysis of toxicity. Table 4 shows the number of combined severe, life-threatening, and fatal toxic reactions for each treatment. There were significantly more severe and life-threatening complications with CAVP16 (P = < 0.0001). Using a logistic model, it was found that this was not due to the imbalance in prior surgery. All five fatal complications on CAVP16 were related to leukopenia and infection as was one of the three fatal reactions to MV. The other two fatalities on MV were due to renal failure (one patient) and interstitial pneumonitis (one).

DISCUSSION

Effective chemotherapy for metastatic non-small cell bronchogenic carcinoma remains elusive. The CAVP16 regimen was chosen to exploit the use of the then newly available agent etoposide. This drug had already demonstrated significant activity against small cell carcinoma (18,19) and some phase II activity against non-small cell lung cancer (20). The Day 1 to Day 8 cycling may not have been optimal (21) but it was felt to be sufficiently similar to a Days 1, 3, and 5 regimen and more convenient for patients. The regimen had been tested earlier by the Albany group (10) with the doxorubicin at 40 mg/m^2 and was found to have a 19% response rate with marked hematologic toxicity. The dose of doxorubicin was reduced to 30 mg/m^2 for this trial in light of the toxicity seen. Mitomycin had shown a 19% response rate in patients with squamous cancer in generation II of EST 2575 (8) and a report from the Miami Veterans Administration (11) suggested activity for MV.

Neither regimen had a significant impact on response or survival, although the patients who responded appeared to be benefited, a pattern seen in earlier studies (7–9). Neither regimen can be recommended for routine usage.

The final point we wish to make concerns the common practice of comparing survival for patients who respond to therapy as opposed to those who do not. These comparisons are ordinarily made to demonstrate the benefits of the therapy for a particular subset of patients (responders) and this report does suggest that this difference may be of some small value to the patient. However, this practice and interpretation are subject to a number of operational and conceptual pitfalls (21,22). While the concept that a response to therapy is responsible for improved survival in a patient with metastatic lung cancer is clinically logical, there are other possible interpretations. First, the drug therapy may simply be acting as a "marker" to identify those patients who were destined to live longer regardless of the effect of therapy. By selecting for a currently unrecognized prognostic factor, it may identify those patients in the tail of the survival distribution. Second, to observe a response a patient must live long enough to have developed a response. Patients who die of organ failure or non-disease-related events prior to the time that most of the responses occurred cannot possibly respond themselves and serve to bias the survival of the nonresponders. Because the therapy in this study was associated with nontrivial toxicity, it is important that unwarranted conclusions about the effect of therapy on survival not be drawn.

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Human Leukocyte Interferon and Intermittent High-Dose Melphalan-Prednisone Administration in the Treatment of Multiple Myeloma: A Randomized Clinical Trial From the Myeloma Group of Central Sweden[1,2]

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In a randomized clinical trial intermittent high-dose melphalan/prednisone (MP) treatment has been compared with human leukocyte interferon (IFN) administration. Three to 6 × 10^6 IU of IFN was given im daily. Therapy was continued to progression of the disease. Fifty-five patients were randomized to receive MP and 75 were randomized to receive IFN. Forty-four percent of the patients receiving MP responded to therapy as opposed to only 14% of the patients receiving IFN ($P < 0.001$). This difference was mainly due to a low response rate in IFN-treated IgG myelomas, while the response rate for IgA and Bence Jones myelomas did not differ significantly between the two treatment groups. Median duration of response was shorter for IFN-responding patients (23 months) as compared to patients in the MP group (35 months). During follow-up, second-line therapy was given more frequently in the IFN group (91%) than in the MP group (59%). Time on initial treatment was significantly shorter in the IFN group (3 months) than in the MP group (19 months). Since more patients responded to second-line therapy in the IFN group than in the MP group, total survival did not differ significantly between the two groups. [Cancer Treat Rep 68:1331–1338, 1984]

Treatment with melphalan as a single drug or in combination with prednisone has improved both quality of life and prognosis in patients with multiple myeloma. Thirty percent to 60% of the patients respond to therapy according to various investigations. The overall median survival time varies between 24 and 36 months, and the median survival time of responders is > 3 years, usually about 5 years (1–6). Different multiple drug combinations have been tested to increase the therapeutic efficacy (5). Whether such protocols yield results superior to melphalan-prednisone (MP) treatment is controversial.

In search for better therapeutic modalities, the clinical effects of human leukocyte interferon of α-type (IFN) have been studied by the Myeloma Group of Central Sweden (MGCS). IFN has been shown to exert antitumoral effects in both animals and humans (7–11). In pilot studies, eight previously untreated patients with myeloma and six patients with myeloma in relapse received 3 × 10^6 IU of IFN as single therapy (12–14). Objective tumor regression was noted in five untreated and in two previously treated patients.

Based on these promising results, a prospective randomized trial was initiated to compare IFN as a single drug with intermittent high-dose MP for the primary treatment of myeloma. The study was started in November 1978. The results of the present report are based on the follow-up of 55 patients treated with MP and of 75 patients treated with IFN.

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MATERIALS AND METHODS

Study Population

Six hospitals participated in the trial. All newly diagnosed and untreated patients who fulfilled the diagnostic criteria for myeloma and who were admitted between November 20, 1978, and August 31, 1982, were entered in the study. The follow-up was performed on March 1, 1984. Patients with a concomitant malignancy were excluded. No patients had to be rejected due to inadequate documentation.

Patients were randomized to receive MP or daily im IFN (for treatment schedules *see below*). With the intention to study the therapeutic effect of two dosages of IFN, a three-arm study design was chosen for a certain period (table 1). However, due to a limited supply of IFN at that time, this part of the study could only be carried out for a short time period. The numbers of patients randomized to the different treatment groups according to the various time periods are shown in table 1. At a preliminary follow-up in June 1981, patients with IgG myelomas in the IFN group were found to have a very low response rate. Furthermore, a few patients with stage B disease (creatinine $\geq$ 200 μmols/L) receiving IFN had a rapid deterioration of their kidney function. Consequently, from that day, all patients with IgG myelomas and stage B disease irrespective of the M-component immunoglobulin type were excluded from the study.

A total of 55 patients were randomized to receive MP and 75 were randomized to receive IFN; the difference in the number of patients between the two treatment groups analyzed is mainly due to a three-arm randomization for a limited period (table 1). There were no significant differences in clinical characteristics between the two treatment groups when comparing the three study periods and taking into account the exclusion of subgroups of patients from June 1981. No differences in response rate or survival between patients receiving MP recruited to the study during three different study periods were noted. All MP patients are therefore presented together. Furthermore, no differences in response rate and survival were found between patients receiving 3×10^6 IU and 6×10^6 IU of IFN and between IFN-treated patients entering the study during different periods. Thus, IFN patients are not shown separately. Since the randomization scheme with regard to the numbers of patients became unbalanced, analyses were performed with and without controlling for suspected confounding variables. Both analyses, however, showed similar findings. Thus, results are presented in the most condensed way possible.

Human Leukocyte Interferon of α-Type

IFN was prepared from human blood leukocytes exposed to Sendai virus and enriched to a specific activity of approximately 1×10^6 IU/mg of protein as previously described (15). IFN was obtained from the Finnish Red Cross Transfusion Service or from Prof. K. Cantell, Helsinki, Finland. The antiviral activity was determined by assaying inhibition of plaques induced by the vesicular stomatitis virus in U cells (16) and was expressed in international units by comparison with the international reference preparation 69/19.

Diagnostic Criteria and Clinical Evaluation

The diagnosis of multiple myeloma was established when at least two of the following criteria were met: (*a*) a monoclonal immunoglobulin peak revealed by serum and/or urine electrophoresis together with a subnormal serum concentration of at least one nonmonoclonal immunoglobulin class (IgG, IgM, and IgA); (*b*) > 10% plasma cells in the bone marrow; and (*c*) osteolytic and/or osteoporotic bone lesions compatible with multiple myeloma (17).

The following laboratory tests were performed at the time of diagnosis: agarose electrophoresis of serum and of 100× concentrated urine; determination of the monoclonal immunoglobulin class by electrophoresis and immunofixation; serum IgG, IgM, and IgA concentration by the Mancini method (18) or by the rocket electrophoresis method according to Laurell (19); quantification of 24-hour urinary protein excretion with the Biuret technique (20); roentgenographic examinations of the skull, vertebral column, and pelvic bones; and bone marrow specimens obtained by aspiration biopsies. The percentage of plasma cells was determined by counting at least 500 cells in separate view fields of stained smears.

The initial evaluation further included erythrocyte sedimentation rate per 1 hour; hemoglobin concentration; wbc count with differential; platelet and

TABLE 1.—No. of patients randomized to the various treatment groups during the 3 different time periods

Study period	MP	IFN	
		3×10^6 IU	6×10^6 IU
Nov 20, 1978–Jan 30, 1980	33	33	None
May 15, 1980–Sept 14, 1980	5	9	9
Sept 15, 1980–Aug 31, 1982*	17	24	None

* All IgG myelomas and all patients with stage B disease were excluded from June 1, 1981.

reticulocyte counts; serum albumin; calcium; creatinine; uric acid; and liver enzymes. The patients were staged according to the system proposed by Durie and Salmon (21).

Treatment Schedules

Patients randomized to MP therapy received 0.25 mg of melphalan/kg of body weight/day and 2 mg of prednisone/kg/day orally during 4 consecutive days. The course was repeated every sixth week (1,2). Treatment was to be deferred or reduced if the neutrophil count was $< 1.5 \times 10^9$/L and/or the platelet count was $< 100 \times 10^9$/L. The prednisone dosage was reduced only if side effects could be attributed to the steroid component. Patients allotted to IFN received 3 or 6×10^6 IU daily by im injections. IFN treatment was to be temporarily deferred when the neutrophil count was $< 1.5 \times 10^9$/L and/or the platelet count was $< 100 \times 10^9$/L. Therapy was continued until disease progression. MP-treated patients with progressive disease (*see below*) changed to high-dose intermittent cyclophosphamide-prednisone every fourth week (2) and patients receiving IFN were given MP treatment.

Local radiotherapy (30 Gy) employing maximally three separate fields was allowed for relief of pain. Hypercalcemia was treated with hydration, furosemide, and, if clinically indicated, prednisone until normalization of serum calcium level. Spinal cord compression with neurological manifestations was usually treated with surgical decompression within 36 hours.

Follow-up

Every 4–6 weeks, or more frequently if clinically indicated, the following laboratory tests were made: erythrocyte sedimentation rate; hemoglobin concentration; wbc count with differential; platelet and reticulocyte counts; serum albumin; calcium and creatinine; electrophoresis of serum and urine; serum concentrations of IgG, IgM, and IgA; and 24-hour urinary protein excretion. Differential counts of bone marrow smears were performed every third month. Skeletal radiograms were repeated every sixth month.

Criteria for Response to Treatment

Clinical response was defined mainly according to the criteria proposed by the Committee of the Chronic Leukemia Myeloma Task Force (2,22). These criteria were: (*a*) decrease of serum M-component concentration by > 50% of the pretreatment value; (*b*) decrease of urinary light-chain excretion to < 0.2 g/24 hours; and (*c*) a > 50% reduction of plasmacytoma size. In addition, patients were classified as responders only if their hemoglobin level was ⩾ 90 g/L, their serum albumin level was ⩾ 30 g/L, and their serum calcium level was ⩽ 2.6 mmols/L.

Criteria for Progression and Relapse

Progression or relapse was attained when one or more of the following criteria were fulfilled: (*a*) increase of the serum M-component concentration with at least 25% of pretreatment or response value, respectively; (*b*) 100% increase in Bence Jones protein 24-hour urinary excretion of pretreatment or response value, respectively; (*c*) serum calcium ⩾ 3 mmols/L, and (*d*) progression of osteolytic lesions.

Statistical Methods

Student's t test was used for testing differences between means. Differences in distribution were tested with the chi-square method (23) or the Fisher's exact probability test. Survival curves were plotted according to the life-table method (24) and the log-rank test was used to test differences in survival (25).

RESULTS

Fifty-five patients were randomized to receive MP and 75 were randomized to receive IFN treatment (table 1). The distribution of age, clinical stage, and M-component immunoglobulin class was similar in the two groups (table 2). There was also no difference found with regard to

TABLE 2.—Pretreatment characteristics*

	MP group (n = 55)	IFN group (n = 75)
Clinical stage		
I	6 (11%)	5 (7%)
II	21 (38%)	32 (43%)
III	28 (51%)	38 (50%)
Male/female	34/21	37/38
Age (yrs)†		
All patients	69 ± 10	68 ± 9
Clinical stage		
I	69 ± 9	64 ± 7
II	67 ± 9	67 ± 9
III	69 ± 10	68 ± 8
Hemoglobin (g/L)†	108 ± 25	105 ± 23
Serum albumin (g/L)†	35 ± 6	35 ± 6
Serum calcium (mmols/L)†	2.53 ± 0.47	2.51 ± 0.43
Serum creatinine (μmols/L)†	148 ± 164‡	135 ± 144§
Patients with serum creatinine < 200 μmols/L	48 (87%)	69 (93%)
M component		
IgG	26 (47%)	44 (59%)
IgA	17 (31%)	16 (21%)
Bence Jones only	12 (22%)	15 (20%)

* Unless otherwise specified, values = No. of patients.
†Mean ± SD.
‡Range, 56–997, μmols/L.
§Range, 45–967 μmols/L.

TABLE 3.—Clinical stage and response to treatment

Clinical stage	MP group Total No. of patients	MP group No. of responders	IFN group Total No. of patients	IFN group No. of responders	P value
I + II	26	12 (46%)	36	4 (11%)	< 0.01
III	28	12 (43%)	38	6 (16%)	< 0.05
Total	54	24 (44%)	74	10 (14%)	< 0.001

hemoglobin concentration, serum albumin, calcium, or creatinine. The two groups contained only six and five stage I patients, respectively (table 2). The response rate and survival of these patients did not differ from those of stage II patients within the same treatment group (data not shown). Stage I and II patients have therefore been pooled in this presentation.

The response to primary treatment could be evaluated in 54 patients (98%) in the MP group and in 74 (99%) in the IFN group. The overall response rate was much higher in the MP group (44%) than in the IFN group (14%) ($P < 0.001$) (table 3). This finding was independent of the clinical stage. A very low response rate was noted for IgG myelomas in the IFN group (5%) (table 4). However, no statistically significant intergroup difference in the number of responding patients was observed for IgA and Bence Jones myelomas, although there was a tendency to fewer responders among IFN-treated IgA myeloma patients. The median time to response was 4 months (range, 1-43) in the MP group and 3 months (range, 2-9) in the IFN group. The response duration time was longer in the MP group than in the IFN group ($P < 0.05$) (fig 1). For MP-treated IgG myelomas the median response duration was 30 months as compared to 4 months for patients receiving IFN. In responding IgA myelomas in the MP group the median response duration was 24 months, while the corresponding figure for patients in the IFN group was 10 months. The median response duration for responding Bence Jones myeloma patients was 14 months in the MP group and 27 months in the IFN group.

Primary treatment had to be changed much more frequently in the IFN group. Accordingly, 59% of the MP patients as compared to 91% in the IFN group changed to second-line therapy ($P < 0.001$). The median time on initial therapy for all patients in the MP group was 19 months (range, 0-63) as compared to 3 months in the IFN group (range, 0-63) ($P < 0.001$). All except three IFN-treated patients who changed therapy had progressive or severe stable disease not responding to treatment. One patient developed an allergic erythema, probably caused by the interferon preparation, and two patients refused to continue the IFN treatment; none had responded. Changes of therapy were not related to the clinical stage or M-component immunoglobulin class (data not shown).

Overall survival of the patients in the two treatment groups as evaluated by life-table analysis did not differ significantly (fig 2). The estimated probabilities of survival after 1 year were 69% and 60%, respectively.

Stage I and II patients survived longer than stage III patients in the MP group ($P < 0.05$) but not in the IFN group (fig 3). No difference between the treatment groups was noted for stage I-II and III patients, respectively. Moreover, there was no statistically significant difference in survival for IgA or Bence Jones myelomas, respectively (figs 4 and 5). However, IgG myeloma patients in the MP group survived longer than in the IFN group ($P < 0.05$) (fig 6). The hematological side effects of treatment are shown in table 5. The hematological toxicity of the MP therapy was assessed immediately prior to the subsequent course. Dose reduction due to myelodepression was necessary in nine patients in this group. One patient in the IFN group died from agranulocytosis and pneumonia after 4 weeks of treatment despite early withdrawal of the drug. No reduction of IFN dosage was considered necessary in the other patients showing signs of myelodepression.

Other drug-related side effects are shown in table 6. Ten patients (18%) in the MP group and 26 patients (35%) in the IFN group had one or more nonhematological symptoms which were considered to be related to the treatment. Initial pyrexia during the first week after IFN injections was not recorded since it may be regarded as "normal" reaction. Itching erythema in one IFN patient prompted withdrawal of the drug. In other patients

TABLE 4.—M-component immunoglobulin class and response to treatment

M-component immunoglobulin class	MP group Total No. of patients	MP group No. of responders	IFN group Total No. of patients	IFN group No. of responders	P value*
IgG	26	13 (50%)	44	2 (5%)	< 0.001
IgA	16	8 (50%)	16	5 (31%)	NS
Bence Jones only	12	3 (25%)	14	3 (21%)	NS

* NS = not significant.

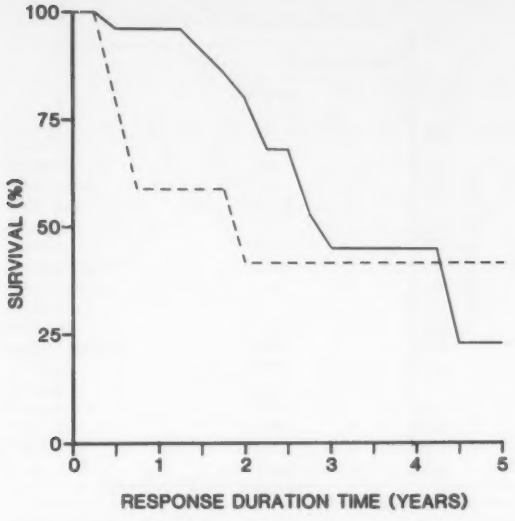

FIGURE 1.—Actuarial response duration time. MP (———) (n = 24) and IFN (---) (n = 10) ($P < 0.05$).

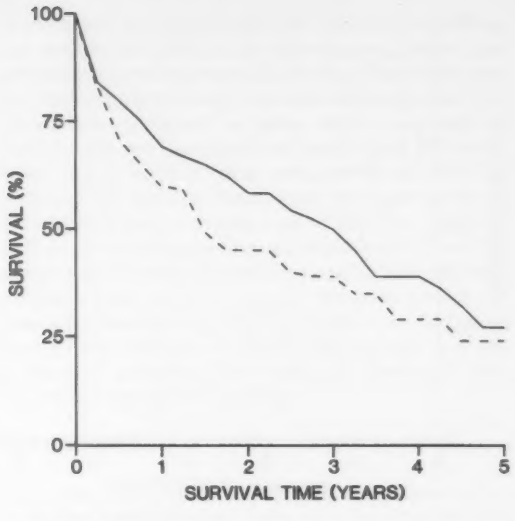

FIGURE 2.—Actuarial survival time. MP (———) (n = 55) and IFN (---) (n = 75) ($P > 0.05$).

erythema gradually disappeared without discontinuing the IFN administration. Increases in serum liver enzymes (alanine and asparatate aminotransferase, alkaline phosphatase) were mild. The IFN therapy had to be temporarily deferred for a short period only in a few patients.

DISCUSSION

This investigation demonstrates that 3×10^6 (or 6×10^6) IU of IFN given as single treatment on a daily basis by the im route induces responses in 14% of previously untreated patients with myeloma. The response rate is clearly inferior to that obtained by intermittent high-

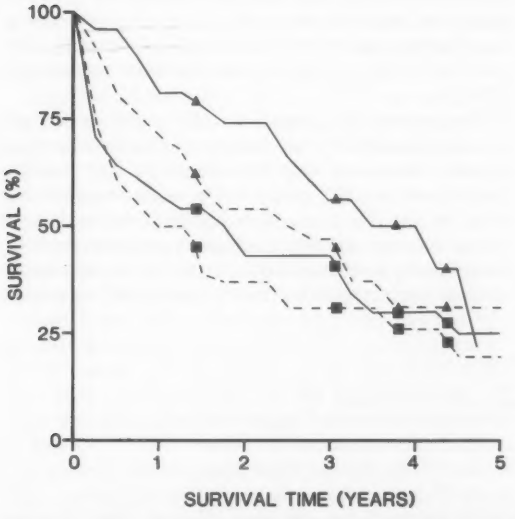

FIGURE 3.—Actuarial survival time in relation to clinical stage. Stage I and II MP (▲———▲) (n = 27) and stage III MP (■———■) (n = 28) ($P < 0.05$). Stage I and II IFN (▲---▲) (n = 37) and stage III IFN (■---■) (n = 38) ($P > 0.05$).

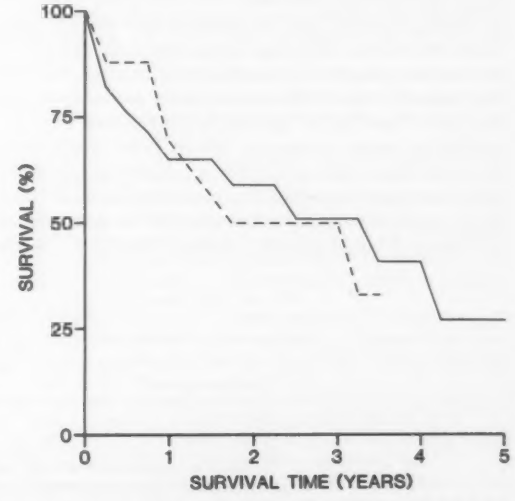

FIGURE 4.—Actuarial survival time for IgA myelomas. MP (———) (n = 17) and IFN (---) (n = 16) ($P > 0.05$).

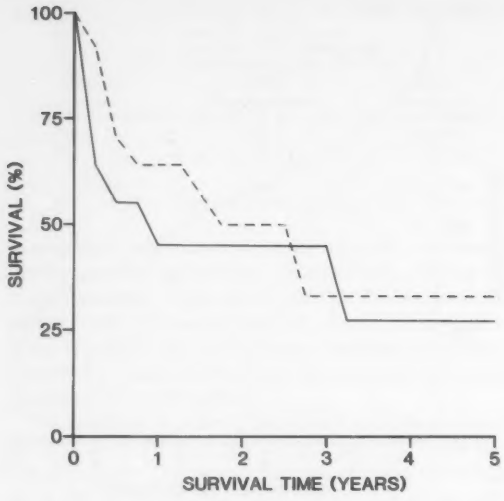

FIGURE 5.—Actuarial survival time for Bence Jones myelomas. MP (———) (n = 12) and IFN (---) (n = 15) ($P > 0.05$).

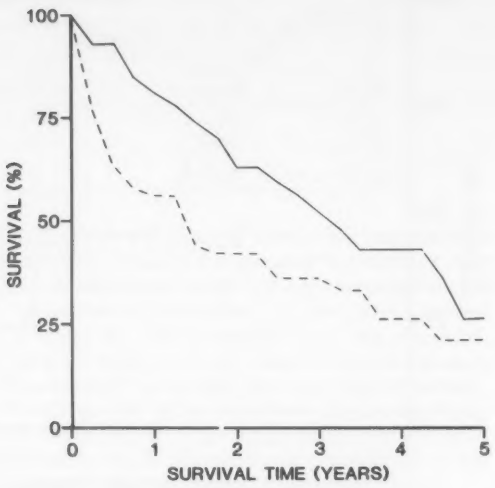

FIGURE 6.—Actuarial survival time for IgG myelomas. MP (———) (n = 27) and IFN (---) (n = 45) ($P < 0.05$).

dose MP treatment. The higher response rate for leukocyte interferon applied according to similar schedules in other studies is most probably due to small materials and selected patients (9,13,14,26). Response was independent of clinical stage but probably related to the myeloma immunoglobulin type. Thus, IgG myelomas rarely responded to IFN therapy, while the response rate for IgA and Bence Jones myelomas seemed to be higher. In fact, the response rate in either of these two groups did not differ significantly from that of the MP group. It should be noted, however, that the response rate for IgA myelomas treated with IFN was 31% as compared to 50% for MP-treated patients. As judged from the small material the response rate of MP-treated Bence Jones myelomas was lower than that of IgA and IgG myelomas. IFN also seemed to induce a response rate in Bence Jones myelomas similar to that in MP therapy. However, the material is too small to exclude the possibility that IFN has a lower response-inducing effect than MP in Bence Jones myelomas. A larger patient material is required to settle the question of myeloma immunoglobulin type and response to IFN treatment.

The median response duration time was shorter in IFN-treated patients (23 months) as compared to patients receiving MP therapy (35 months). The shortest observed response duration time in the IFN group was 3 months but response may last for > 3 years. It should be realized that IFN therapy was continued during the remission phase and was not withdrawn until disease progression. Long-lasting remissions have also been reported by Alexanian et al (26) in a few myeloma patients treated with IFN.

Total survival of patients initially receiving IFN was not significantly different from that of patients receiving primary treatment with MP. However, IgG myeloma patients in the IFN group had a worse survival than those in the MP group. It is highly probable that the poorer survival of IFN-treated IgG myelomas reflects the significantly lower response rate which is strongly associated with survival (2). Secondary therapy (MP treatment)

TABLE 5.—Hematological toxicity

Treatment group	No. of patients with toxicity grade—* I	II	III
MP (n = 55)	14	4	6
IFN (n = 75)	14	15	3†

*Grade I = leukopenia, 2-3 × 10^9 cells/L and/or thrombocytopenia, 100-150 × 10^9 cells/L; grade II = leukopenia, 1.5-2 × 10^9 cells/L and/or thrombocytopenia, 50-100 × 10^9 cells/L; and grade III = leukopenia, < 1.5 × 10^9 cells/L and/or thrombocytopenia, < 50 × 10^9 cells/L.

†1 patient with lethal agranulocytosis.

TABLE 6.—Nonhematological toxicity

	No. of patients (%)	
	MP group (n = 55)	IFN group (n = 75)
Fever*	—	1 (1%)
Shivering	—	2 (3%)
Perspiration	—	3 (4%)
Feebleness	6 (11%)	13 (17%)
Headache	—	1 (1%)
Vertigo	1 (2%)	3 (4%)
Taste anomaly	1 (2%)	3 (4%)
Impaired vision	—	2 (3%)
Depression	—	2 (3%)
Hair loss	5 (9%)	4 (5%)
Itching erythema	4 (7%)	4 (5%)
Stiffness	2 (4%)	1 (1%)
Myalgia	2 (4%)	—
Nausea	1 (2%)	8 (11%)
Vomiting	1 (2%)	4 (5%)
Diarrhea	1 (2%)	2 (3%)
Liver injury	1 (2%)	6 (8%)

*Fever was common during the 1st week of IFN administration. Recorded here is only fever on subsequent treatment. 26 patients (35%) receiving IFN had nonhematological side effects compared to 10 (18%) in the MP group.

in the IFN group had, however, a good therapeutic effect. When these results became evident patients with IgG myelomas were excluded from the study. At the same time we also observed that the kidney function rapidly deteriorated during IFN therapy in some patients who had shown evidence of renal insufficiency prior to treatment. Hence, all patients with stage B disease were also excluded. However, no explanation for the rapid decline in renal function in these patients is available but Gresser et al (27) have described the development of nephritis in IFN-treated newborn mice.

Although a dose of 3×10^6 IU of IFN was well-tolerated, some side effects were noted. The frequency and types of side effects are similar to those reported by others (12,28-30). The assumption that normal cells can be affected by these IFN doses is supported by the development of myelosuppression and alopecia in some patients. Inhibitory effects of IFN on myeloid colony formation and in vitro differentiation of granulocytes have also been shown (31,32). One of our patients developed lethal agranulocytosis. Although these effects can only tentatively be ascribed to IFN treatment, a number of cases of granulocytopenia in IFN-treated patients have been reported (9,30,33). The mechanism for the slight and reversible elevation of liver enzyme values is unclear.

In several reports human leukocyte IFN and fibroblast IFN have been shown to have some antitumoral effects in patients with multiple myeloma (9,12-14,26,28,34). However, as demonstrated here, IFN used daily in doses of $3\text{-}6 \times 10^6$ IU induced objective responses in myeloma patients but the therapeutic effect was clearly inferior to conventional cytostatic treatment. This conclusion does not exclude that IFN used in other dosages, routes, and schedules may be active as a single drug or as an adjuvant to other drugs in the treatment of multiple myeloma. It is particularly important to better define subgroups of patients who are most likely to benefit from IFN treatment. A pilot study is being conducted by the MGCS in patients with IgA and Bence Jones myelomas (stage A) who initially receive a daily dose of $20\text{-}30 \times 10^6$ IU of IFN and a maintenance dose of $10\text{-}20 \times 10^6$ IU of IFN given intermittently. Preliminary results have shown a response rate of 50% (seven responses among 14 patients) (data to be published). Furthermore, high-dose recombinant leukocyte A IFN (33,35) may also be of importance in attempts at increasing the therapeutic efficacy of IFN therapy for myeloma.

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Efficacy Trial of Pipobroman in Essential Thrombocythemia: A Study of 24 Patients[1]

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Pipobroman (PB) was tested in a prospective efficacy trial in 24 previously untreated patients with essential thrombocythemia and followed up for a median of 47 months (range, 12–120). Plateletpheresis was not done. Hematologic complete response (platelet count $< 450 \times 10^9$/L for 3 consecutive months) was achieved in 92% of the cases, in a median time of 12 weeks (range, 2–22). However, continuous low-dose maintenance therapy with PB was necessary to avoid recurrences of the disease. Major thrombocythemia-related complications were observed in 20% of the cases; the 5-year overall and complication-free survival rates were 92% and 75%, respectively. No acute or chronic toxicity was observed; no drug-induced amenorrhea and subsequent acute leukemia occurred. PB does appear to be efficacious in essential thrombocythemia; however, the evaluation of its leukemogenic risk will await further experience. [Cancer Treat Rep 68:1339–1342, 1984]

Essential thrombocythemia (ET) is a clonal disorder of hematopoietic tissues (1), with prevalent megakaryocytic hyperplasia in bone marrow and marked thrombocytosis in peripheral blood. Recurrent hemorrhages, thrombotic events (2–5), and neurologic symptoms (6) are the most frequent complications of this disease. To avoid these complications, an attempt to keep the platelet count within the normal range by myelosuppression seems reasonable; however, only one randomized study by the Polycythemia Vera Study Group (PVSG) has focused on the efficacy of myelosuppressive therapy in ET (7). In the PVSG trial, melphalan was randomized versus radiophosphorus (^{32}P), and the response rate at 3 and 6 months was significantly higher for melphalan than for ^{32}P. The toxicity with either regimen was negligible.

Pipobroman (PB), a neutral amide of piperazine, was shown to have antineoplastic activity against certain animal tumors, including transplanted adenocarcinoma Ca 755 in mice and spontaneous breast adenocarcinoma in rats (8). Its chemical structure suggests that it is an alkylating agent although unrelated to the usual HN2 drugs (9). In clinical studies PB was effective in polycythemia vera (PV) (10,11), with about an 80% response in patients refractory to ^{32}P and other alkylating agents. Its toxicity was shown to be strictly dose-related and consisted of bone marrow depression of variable degree; side effects were infrequent and consisted of anorexia, nausea, vomiting, and abdominal cramps (10,11).

In 1970 and 1973, we started two prospective studies to test the efficacy of PB in PV and in ET, respectively, and to evaluate any possible acute and long-term complication of this therapy. The data obtained in PV have been published recently (12). In this paper we present our results for ET.

PATIENTS AND METHODS

This study includes 24 consecutive patients with ET admitted to the Division of Hematology from January 1973 through December 1982. After all the possible causes of secondary thrombocytosis were ruled out, the diagnosis of ET was based on the following criteria: (*a*) platelet count $> 750 \times 10^9$/L at three separate determinations for 6 consecutive months; (*b*) megakaryocytic hyperplasia in bone marrow biopsy without fibrosis or myeloid metaplasia of any entity; and (*c*) absence of the Philadelphia chromosome. The erythrocyte mass was calculated in eight patients, when the hemoglobin value was > 15 g/dl

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(males) or 14 g/dl (females). No patient with previous splenectomy was considered in this trial. Table 1 shows the main characteristics of patients and the symptoms at diagnosis. Male to female ratio was 0.6, and the median age was 50. Four patients were < 30 years old and were considered to have juvenile ET. The mean platelet count was 1170 × 10^9/L, and the mean wbc count was 12.5 × 10^9/L; leukocytosis (wbc count > 10 × 10^9/L) was present in 14 patients (59%) and erythrocytosis was present in eight (33%). The mean hemoglobin and hematocrit values for the whole group were 14.2 g/dl and 42%, respectively. Splenomegaly was found in nine patients (37%), hepatomegaly in 12 (50%), and concomitant hepatosplenomegaly in six (25%). No chromosomal abnormalities were found at diagnosis of ET. Main symptoms included paresthesias in hands or feet (46%), with or without acrocyanosis, headache (17%), dizziness (12%), angina (8%), and pruritus (8%). Thrombotic manifestations consisted of venous thrombosis (21%), transient cerebral ischemia, including an episode of amaurosis fugax (17%), peripheral arteriopathy (8%), and myocardial infarction (4%). Hemorrhagic events included gastrointestinal bleeding and bleeding after dental avulsion (three patients). Five patients had a previous history of venous thrombosis (two having had splenic thrombosis); two patients were relatives (mother and daughter), and two had a family history of other hematologic neoplasias (acute lymphocytic and chronic lymphocytic leukemia, respectively). Concomitant nonneoplastic pathology included hypertension in three cases and diabetes mellitus in one.

PB was given as a single agent. The induction of response consisted of daily administration at a dose of 50 mg orally, with a weekly peripheral blood cell count. No plateletpheresis was done, either before therapy with PB or during the course of the disease. Hematologic complete response to PB was defined by a reduction of the platelet count to < 450 × 10^9/L for at least 3 consecutive months, without concomitant stable anemia or leukopenia. Resistance to PB was defined by the absence of any decrease in the platelet count (< 750 × 10^9/L), after 2 months of continuous therapy at 50 mg/day and 1 additional month at 75 mg/day. At remission, continuous maintenance was started with PB at a dose of 25 or 12.5 mg/day, according to the platelet count. Relapse was defined by an increase in the platelet count to > 750 × 10^9/L during maintenance therapy or after PB treatment was stopped. The therapy was withheld when platelets were < 250 × 10^9/L and/or after 18 months of stable platelet count < 450 × 10^9/L. In addition to PB, dipyridamole (225 mg/day) was given when the platelet count was > 1000 × 10^9/L. The median follow-up was 47 months (range, 12–120), with physical and hematologic examination every 8 weeks.

Thrombocythemia-related complications and endpoints in the analysis of the complication-free time were considered the major hemorrhagic and/or thrombotic episodes. Life-table analysis was employed to estimate the overall and complication-free survival.

TABLE 1.—Patient characteristics and symptoms at diagnosis*

Characteristics	No. of patients	%
Total	24	
Male	9	37
Female	15	63
Age		
< 30	4	17
30–70	17	71
> 70	3	12
Leukocyte count (× 10^9/L)		
4–10	10	41
> 10	14	59
Platelet count (× 10^9/L)		
750–1000	14	59
1000–1500	6	25
1500–2000	1	4
> 2000	3	12
Hemoglobin (g/dl)		
< 10	2	8
10–15	14	59
> 15	8	33
Hepatomegaly	12	50
Splenomegaly	9	37
Symptoms		
Paresthesias	11	46
Hemorrhages	6	25
Venous thrombosis	5	21
Transient ischemic attack	4	17
Headache	4	17
Dizziness	3	12
Peripheral arteriopathy	2	8
Angina pectoris	2	8
Pruritus	2	8
Myocardial infarction	1	4

*See the Patients and Methods section for details.

RESULTS

Hematologic complete response was achieved in 22 of 24 (92%) previously untreated patients who were given PB as a single agent. Complete resistance to PB was observed in two cases only (patients who had received 50 mg/day for 2 months and 75 mg/day for 1 additional month). The median time required from the initiation of therapy to a platelet count < 450 × 10^9/L was 12 weeks (range, 2–22). In three of nine patients with initial splenomegaly, the spleen remained palpable below the costal margin, while platelets were within the normal range. No relation was found between the initial platelet count and the time required for response. In responders, the median platelet count was 345 × 10^9/L, the median wbc count was 7.7 × 10^9/L, and the median hemoglobin level was 14 g/dl. Wide fluctuations in platelet count from 200 to 700 × 10^9/L were observed in ten patients during maintenance therapy. The mean duration of main-

tained response was 15 months (range, 2-72+).

To date, 13 patients have relapsed in spite of maintenance therapy; they have responded to an increased dose of PB (75 mg/day). Eight patients stopped PB after 18 months with platelet counts $< 450 \times 10^9$/L (stable disease). Five of these patients (21% of the total cases) are in a stable phase without therapy after 2 years (long-remitters). No apparent relation was found between long remission and age or duration of induction therapy. The median duration of induction therapy in long-remitters was 10 weeks. Subjective disturbances were not experienced after PB, except for mild nausea in four patients. Leukopenia was observed in one patient. No drug-induced amenorrhea was seen in eight menstruating females.

Major thrombocythemia-related complications were seen in five patients during therapy with PB: gastrointestinal bleedings (two patients), cerebral thrombosis (one), retinal thrombosis (one), and progressive anemia without bleeding (one). One patient has died of a stroke after 12 months of follow-up and a very short-lasting response to PB. The 5-year actuarial probability of survival was 96% and of freedom from major complications was 75%. No subsequent solid tumor, acute leukemia, or transformation into other forms of myeloproliferative diseases were observed in the cumulative 110 person-years-at-risk.

DISCUSSION

In this trial, PB proved to be an efficient drug in 92% of the previously untreated patients with ET, allowing a reduction of the platelet count to within the normal range in a median of 12 weeks, without acute or chronic toxicity. PB efficacy in ET was comparable to that reported by the PVSG for melphalan (7). The major thrombocythemia-related complications were also minimal during therapy. The response rate and the mode of action of PB in ET were quite similar to those observed in PV in our series (12). In particular, the response rate to PB was > 90% in both diseases and the time required for the hematologic response was rather long (about 3 months). A continuous low-dose maintenance therapy was required to avoid overt recurrences of the disease, and a strict dose-effect relation was observed during the maintenance therapy. Long-remitters after stopping therapy represented 20% of the total cases and did not show particular characteristics at diagnosis. At disease progression, patients who had become refractory to low-dose PB usally responded to an increased dose of the same drug (up to 100 mg/day); an absolute resistance to PB was seen in < 10% of the patients.

In regard to chronic toxicity, it is encouraging that in this series no drug-induced amenorrhea occurred in eight menstruating females, nor were there cases of subsequent acute leukemia. However, the piperazines are alkylating agents and several of them are carcinogenic in animals (13,14). An increased actuarial risk of leukemia has been observed by the PVSG during chlorambucil treatment in PV (15). In our parallel trial with PB in PV (12) we found a 6% and 9% actuarial risk of acute leukemia at 5 and 7 years. The potential leukemogenic risk of PB, even in ET, remains the major issue, since this is a rather small series of patients who have been followed for a maximum of 10 years.

The necessity of a chronic maintenance in ET and the possibility of long-term complications are of particular concern in younger patients, in whom the disease seems to be more benign and chemotherapy not necessary unless serious complications develop (16). Even though our policy in ET is not to treat young patients, the present series included four patients < 30 years of age who presented with important symptoms: severe hemorrhages (two patients), venous thrombosis (one), and very high platelet count and severe paresthesias (one).

In conclusion, PB is effective in ET but requires continuous maintenance therapy. A longer follow-up and observation on a larger scale are needed to assess the risk of leukemia in ET during long-term therapy.

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Combination Chemotherapy for Advanced Non-Hodgkin's Lymphomas Other Than Diffuse Histiocytic or Undifferentiated Histologies[1]

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A combination chemotherapy program using methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone (M-BACOD), which resulted in a high complete response rate and prolonged disease-free survival in lymphomas of the unfavorable diffuse histiocytic and diffuse undifferentiated histopathologic subgroups, was administered to 44 patients with advanced favorable and intermediate-prognosis non-Hodgkin's lymphomas, including nodular lymphoma of the poorly differentiated lymphocytic, mixed, and histiocytic subtypes, and diffuse lymphoma of the poorly differentiated lymphocytic or mixed histologic subtypes. High-dose methotrexate (3 g/m^2) was given on Day 14 between cycles of bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone, administered every 3 weeks for ten cycles. Leucovorin factor (10 mg/m^2) was given iv 24 hours after the methotrexate infusion was completed, and was continued at 10 mg/m^2 by mouth every 6 hours for 72 hours. Therapy was well-tolerated, with predictable myelosuppression in the majority of patients. The complete response rate was 57% (25 of 44 patients), including ten of 18 (56%) patients with nodular and 15 of 26 (58%) patients with diffuse lymphomas. Median overall follow-up among living patients is 65 months, 58 months for patients with nodular and 69 months for patients with diffuse histologic subgroups. Overall survival at 5 years was 64% for patients who achieved complete response, 32% for partial responders, and 0% for those patients who did not respond. Disease-free survival of complete responders was 43% at 5 years, with only one disease-related death noted after 36 months. The nodular and diffuse patient subgroups had similar overall and disease-free survivals. Although initial bone marrow involvement was documented in nine of 18 (50%) nodular patients and in 13 of 26 (50%) diffuse patients, CNS relapse occurred in only one complete and two partial responders. The prolonged disease-free survival observed after M-BACOD therapy demonstrates that durable responses can be achieved with intensive chemotherapy. [Cancer Treat Rep 68:1343–1350, 1984]

Histopathologic subgroups of the non-Hodgkin's lymphomas that have a long natural history even in advanced stages have been identified and have been called favorable prognosis lymphomas. They include nodular poorly differentiated lymphocytic lymphoma (NPDL) and nodular mixed lymphocytic lymphoma (NM) within the Rappaport classification of non-Hodgkin's lymphoma (1,2). Most of these patients will in fact have advanced disease (60%–85% clinical stage III or IV and 90% pathological stage III and IV) (3–6). Whether deferment of therapy (7–9) is superior to single alkylating agent therapy (10–13), combination chemotherapy (10–20), whole-body irradiation (12,21–24), combination chemotherapy and total lymphoid irradiation (11,12), or combined chemotherapy and whole-body irradiation (22) in terms of response rate, remission duration, and survival of

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these patients remains unsettled. This is mainly due to a sustained tendency to relapse regardless of therapeutic approach.

Patients with advanced-stage diffuse poorly differentiated lymphocytic lymphoma (DPDL) or diffuse mixed lymphoma (DM), as well as those with nodular histiocytic lymphoma (NH) Rappaport histopathological subgroups of non-Hodgkin's lymphomas, have prognoses intermediate between those for favorable nodular histological subgroups (8-year median survival) and those for the unfavorable diffuse histiocytic or undifferentiated lymphomas (< 1 year median survival) (2,17,25-27). Prior to the advent of combination chemotherapy, overall survival of the patients with DM and DPDL at 2 years was 50% (25). More recently, a 75% 3-year survival of these patients was noted after treatment with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or HOP (doxorubicin, vincristine, and prednisone). Disease-free survival of 87% at 3 years for similar patients who completely respond to COPP (cyclophosphamide, vincristine, procarbazine, and prednisone) or COPA (cyclophosphamide, vincristine, prednisone, and doxorubicin) chemotherapy has also been reported (17). In contrast to patients in the favorable prognosis subgroups who have a sustained tendency to relapse, the remission duration curves in some studies tend to plateau for patients in the intermediate prognosis subgroups (ie, NH, DM, and DPDL) (25-27). It remains a matter of debate whether long-term cure has been achieved.

The encouraging results from these select series of patients treated in most instances with combination chemotherapy prompted a trial of our combination chemotherapy program using methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone (M-BACOD) (28) in patients with histologic subgroups of lymphoma other than diffuse histiocytic lymphoma (DHL) or diffuse undifferentiated lymphoma (DUL) who had an advanced symptomatic stage with features predictive of poor prognosis (2). At our institution, this chemotherapy regimen has resulted in a 72% complete remission (CR) rate and projected 65% disease-free survival at 5 years in patients with the unfavorable DHL and DUL histologic subtypes of non-Hodgkin's lymphomas (28).

PATIENTS AND METHODS

Patient Characteristics

A total of 44 consecutive patients with advanced lymphomas other than DHL or DUL were treated with M-BACOD from 1975 to 1981 (table 1). All original tissue sections were reviewed by one hematopathologist (G. S. Pinkus) and classified according to a modified Rappaport system (29). DPDL, the major subgroup in this study (26 patients), was composed of predominantly small cleaved follicular center cells or small lymphoid cells with marked nuclear irregularity. One patient had some morphological characteristics suggestive of lymphoblastic lymphoma (markers unavailable). DM tumors included a spectrum of lymphoid cells, both large and small forms, some with prominent nuclear irregularity, conforming to the nonfollicular center cell type of mixed lymphoma recently described by Nathwani et al (30). Of the 18 nodular patients, nine were considered NPDL by classical Rappaport criteria and three were considered NM due to a predominantly nodular histology. Six cases were NH and consisted of either large cleaved or noncleaved follicular center cells. Surface marker analysis was done as previously described (31) for 17 cases (12 B-cell and five T-cell); B-cell tumors were otherwise presumptively diagnosed on the morphologic basis of predominantly follicular center cells. Patients with DHL (large cell) or DUL have been reported separately (28) and are not included in this report.

TABLE 1.—Clinical characteristics of 44 patients treated with M-BACOD

Characteristic	No. of patients
Males/females	32/12
Median age in yrs (range): 50 (18-75)	
Nodular lymphoma	18
NPDL	9
NM	3
NH	6
Stage	
II	0
III	3
IV marrow	11
IV liver	3
IV lung	1
Diffuse lymphoma	26
DPDL	17
DPDL (T)	2
DM	2
DM (T)	5
Stage	
II	5
III	4
IV marrow	12
IV other	5

Entrance cirteria included (*a*) histological diagnosis of NPDL, NM, NH, DPDL, or DM non-Hodgkin's lymphoma; (*b*) stages II, III, or IV disease as outlined by the Ann Arbor Conference (32); (*c*) a baseline creatinine clearance of > 60 ml/minute; and (*d*) no prior chemotherapy with a doxorubicin-containing regimen or greater than limited-field radiotherapy. No patients with stage I disease were included in this series.

Initial staging procedures have been previously described (20) and include bipedal lymphangiography, and abdominal and pelvic computerized tomographic (CT) scans. Thoracic CT scans were obtained in patients with mediastinal or lung involvement. Bone marrow biopsy

and aspiration were obtained in all patients. Bone survey and scan, as well as gallium scan, were performed in selected symptomatic patients. Exploratory laparotomy was carried out in only three patients when unexplained abdominal problems or masses occurred.

The clinical features of the 44 patients treated with M-BACOD are shown in table 1. There were 32 men and 12 women. Eleven patients had prior therapy, consisting of radiotherapy only (seven), chemotherapy only (three), and radiotherapy and chemotherapy (one). No patients with nodular and five of 26 (19%) patients with diffuse histologies had stage II disease. Overall, 33 patients (75%) had stage III or IV disease. All patients had systemic symptoms and the majority (80%) had bulk disease (mass lesions > 10 cm). The most common extranodal site was the bone marrow (52% of all patients), followed by liver, lung, gastrointestinal, and soft tissue involvement.

Chemotherapy Program

High-dose methotrexate (3 g/m^2) was given on Day 14 between cycles of the myelotoxic drugs bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone, administered every 3 weeks for ten cycles. Five-day courses of dexamethasone (6 mg/m^2) were given by mouth as indicated. Details of methotrexate administration have been previously published (33,34). Urinary alkalinization (pH > 7.0) was achieved by administration of 3 g of sodium bicarbonate by mouth every 3–6 hours the night before methotrexate infusion and for 48 hours thereafter. Hydration with at least 3 L of fluid per day was maintained before, during, and after each course. Methotrexate was administered by iv infusion over 40–60 minutes. Leucovorin factor (10 mg/m^2) was given iv 24 hours after the methotrexate infusion was completed and was continued at 10 mg/m^2 by mouth every 6 hours for 72 hours. If the serum creatinine increased by > 50% above baseline at 24 hours and/or the methotrexate level was $> 5 \times 10^{-6}$ M, leucovorin factor was increased to 100 mg orally every 3 hours until the serum methotrexate level decreased to 1×10^{-8} M.

Blood cell counts were obtained weekly during the course of therapy. Doses of doxorubicin and cyclophosphamide were reduced according to the following schedule: wbc count 2000–3500/mm^3 or platelet count of 50,000–100,000/mm^3, 50% decrease; wbc count < 2000/mm^3 or platelet count < 50,000/mm^3, dosage delayed. Vincristine was reduced by 50% if moderate neuropathy developed and was discontinued with severe neuropathy. Bleomycin was omitted with the first evidence of unexplained clinical or radiographic pulmonary toxicity. Radiotherapy to involved sites was not included in the protocol.

One month after completion of the entire M-BACOD program, baseline staging procedures were repeated. A CR was defined as no evidence of residual disease; a partial response (PR) indicated > 50% reduction in measurable disease for at least 1 month. No response signified progressive disease, transient disease regression lasting < 1 month, or tumor regrowth before the end of M-BACOD treatment.

Statistical Methods

Disease-free survival was calculated from the beginning of CR to the first objective evidence of disease relapse or death. Survival was calculated from the start of treatment until death. Survival and disease-free survival curves are plotted by the life table method (35). Statistical significance was determined by the Wilcoxon test, modified to deal with life table data (36).

RESULTS

Response to Therapy

The overall response rate was 89% (39 of 44 patients); the CR rate was 57% (25 of 44 patients) (table 2). There was no significant difference in the CR rate with age, histology, or stage of disease. Moreover, patients with prior therapy had a 55% CR rate equivalent to that for patients without prior therapy (58%). The CR rates for major sites of stage IV disease were: bone marrow (57%), lung (60%), soft tissue (48%), bone (50%), or liver (44%).

A total of 12 of 25 (48%) patients with CR have relapsed (six of ten nodular and six of 15 diffuse patients). One patient died of other causes without active lymphoma. Five of six relapses in diffuse lymphoma occurred within 6 months of completing M-BACOD therapy; in contrast, only one of six nodular patients recurred within 1 year and three recurred at > 30 months. Relapse occurred in patients with stage II disease (two of four) as frequently as in those with stage III and IV disease (ten of 21). Relapse was not more common at the site of previous bulk disease (> 10 cm) or in patients with bone marrow involvement. In fact, six of the 12 relapses were at sites other than those involved at the start of M-BACOD therapy. Of the six late relapses (20–41 months), three occurred in solitary lymph node sites. There was one CNS relapse and one relapse in bone marrow. Four of the 12 patients who relapsed remained alive at 20 to 47 months after reinduction therapy.

While PR occurred in 14 of 44 patients (32%) (table 2), the median duration of response was only 8 months. Two patients developed CNS relapse. Four of 14 (29%) patients remained alive at 34 to 66 months. Overall, only three of 44 (7%) patients were nonresponders, and two patients (4%) were unevaluable due to early toxic deaths.

Survival

Median follow-up among all living patients is 65

TABLE 2.—Response to treatment with M-BACOD

Response	No. of patients (%) Nodular (N = 18)	Diffuse (N = 26)	Total (N = 44)
CR	10 (56)	15 (58)	25 (57)
PR	7	7	14 (32)
No response	0	3	3 (7)
Inevaluable	1	1	2 (4)

months from the onset of therapy, 58 months for nodular and 69 months for diffuse patients. Overall, 19 of 44 (43%) patients are alive (fig 1). At a median 65-month follow-up time, 60% (15 of 25) of patients with CR remain alive, with only one disease-related death after 36 months; 11 of 25 (44%) are still free of relapse (fig 2). In contrast, only four of those 19 patients (21%) with PR remain alive; neither the three nonresponding patients nor the two patients with toxic deaths lived > 9 months. Forty-two percent of nodular and 44% of diffuse patients remained alive without a statistically significant difference in their survival time (fig 3). However, among patients with CR, those with diffuse lymphocytic lymphoma and T-cell markers have a lower disease-free survival time (17% at 9 months) than patients with diffuse but non-T-cell lymphomas (67% at 5 years) or those with nodular lymphomas (40% at 5 years) ($P < 0.05$; fig 4).

Toxicity

The majority of the 44 patients had predictable myelosuppression due to cyclophosphamide and doxorubicin, with a median wbc count nadir of 2300/mm^3 and a platelet count of 100,000/mm^3. Two patients were hospitalized for fever of undetermined origin and four were hospitalized for nonspecific pneumonitis. One patient developed profoundly disabling vincristine-related neuropathy, necessitating discontinuation of this drug. Bleomycin was discontinued in six patients after a median dose of only 32 mg/m^2 (in one patient due to an immediate bleomycin reaction and in the other five due to probable bleomycin-related pulmonary toxicity). Radiographically demonstrated pulmonary infiltrates were evident in four of these five patients and resolved in all cases. Profound methotrexate-related renal failure, stomatitis, maculopapular rash, and pancytopenia were complicated by sepsis and led to toxic deaths in two patients. The methotrexate dose was decreased in five patients due to the development of elevated creatinine in two cases, and pleural effusion, nephrolithiasis, and renal mass in one case each. Toxic effects related to doxorubicin included the development of clinical conges-

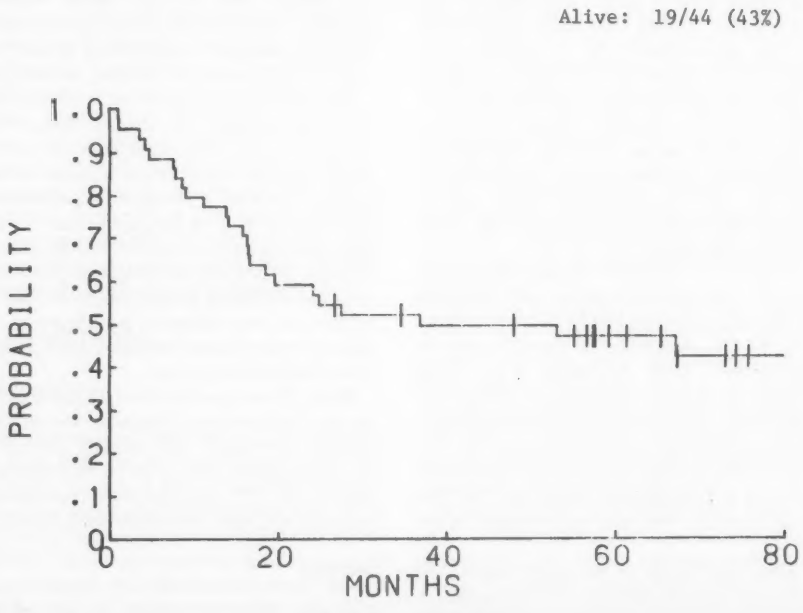

FIGURE 1.—Overall survival.

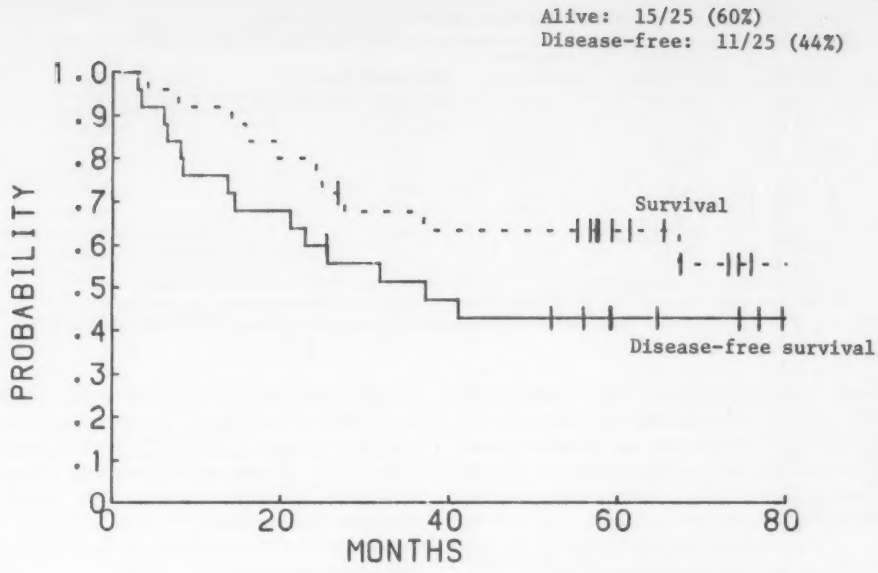

FIGURE 2.—Survival and disease-free survival of complete responders.

tive heart failure in one patient after a total dose of 60 mg/m². In two patients > 70 years of age, drug doses were reduced to 50%. Overall, 13 of 14 (29%) patients required alterations in dose or schedule. Ten patients achieved CR, two achieved PR, and one did not respond, suggesting that these alterations did not influence probability of response.

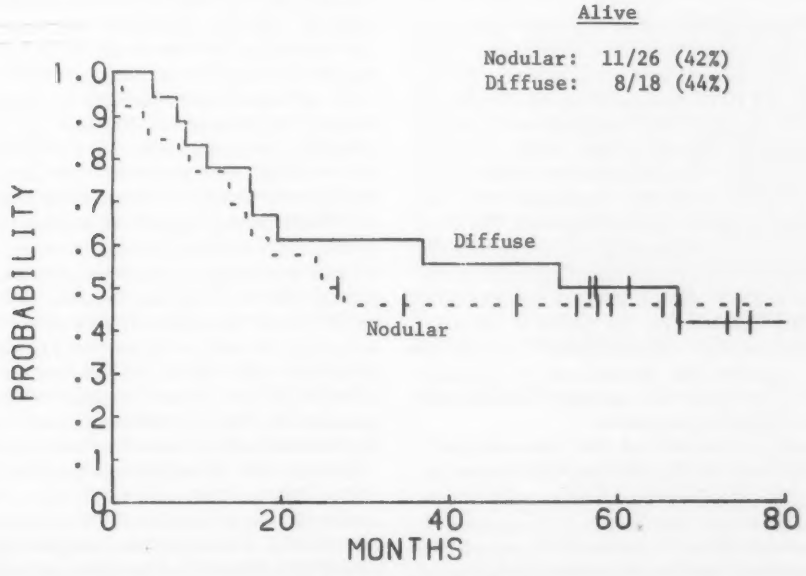

FIGURE 3.—Survival by cell pattern.

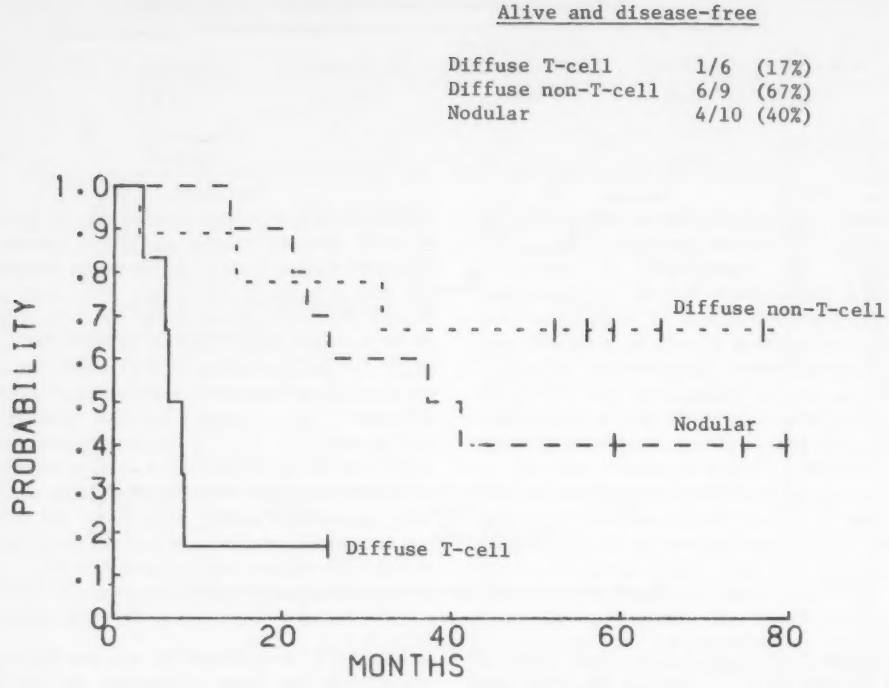

FIGURE 4.—Disease-free survival by histology.

DISCUSSION

The M-BACOD program resulted in a high CR rate and prolonged disease-free survival of complete responders when employed in patients with advanced-stage NPDL, NM, NH, DM, and DPDL histopathological subtypes of non-Hodgkin's lymphomas. This occurred in the setting of systemic symptoms and bulk disease, which would predict for poor prognosis. The estimated 5-year overall and disease-free survival of complete responders (64% and 43%, respectively) suggests durable responses. The short survival of partial or nonresponding patients reflects the aggressive nature of these lymphomas, despite their low- and intermediate-grade histopathological subtypes. Our M-BACOD data, coupled with the results of other reported combination chemotherapy programs (2,17,25-27), supports the selected use of intensive chemotherapy in patients with aggressive intermediate prognosis non-Hodgkin's lymphomas.

Approximately one-half of all non-Hodgkin's lymphomas will have NPDL, NM, and NH histopathologic subtypes (6). In our series, 18 such patients were treated (table 1). Greater than 90% of these patients reportedly have stage III or IV disease (3-6), as was the case in our patients. Various approaches, including no initial therapy (7-9), single alkylating agent therapy (10-13), combination chemotherapy (10-20), whole-body irradiation (12,21-24), and combined chemotherapy and radiation therapy (11,12,22), have been employed to treat these patients (9). As shown in table 3, CR rates, median duration of remission, and median actuarial survival are equivalent regardless of therapy employed. However, in patients with NH histology who have a less favorable prognosis (median survival of 31 months), Osborne et al (27) have reported a 73% CR rate and disease-free survival of 4.5-11+ years after intensive combination chemotherapy, suggesting possible cures. We chose to treat only nodular patients in advanced stage with multiple parameters predictive of poor prognosis. In this setting, M-BACOD therapy resulted in a 56% CR rate (table 2) and a 40% disease-free survival at 5 years (fig 3), comparing favorably with previously reported series. The observation that six of our ten nodular patients who achieved CR have relapsed provides evidence for the aggressiveness of disease in these patients. It remains to be determined whether longer-term benefit will result.

Patients with advanced-stage lymphocytic lymphoma of the DM or DPDL subgroups have a survival time similar to those with nodular histiocytic histology, but inferior to that of other nodular subtypes (2,6). Prior to the advent of combination chemotherapy, patients with DM or DPDL lymphomas had a 50% survival at 2 years, com-

TABLE 3.—Therapy of advanced-stage nodular lymphoma

Therapy*	CR rate (%)	Disease-free survival (mos)	Overall survival (mos)	Ref No.
Single-agent chemotherapy	13–65	12–36	30–96+	10–12
CVP	37–88	6.5+–37+	96+ to 124	10–16
C-MOPP	48–77	16.5–90+	48–101+	14, 17, 18
CHOP	68	6.5+	12+	16
CHOP-Bleo	62	8.3+	8.3+	19
BACOP	89	14+	25+	20
Whole-body irradiation	71–85	12–30	26–96+	12, 21–24
Radiation therapy and chemotherapy	70–75	15–48+	24–96+	11, 12, 22

*CVP = cyclophosphamide, vincristine, and prednisone; C-MOPP = cyclophosphamide, methotrexate, vincristine, procarbazine, and prednisone; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; Bleo = bleomycin; and BACOP = bleomycin, doxorubicin, cyclophosphamide, vincristine, and prednisone.

pared to only 1 year for patients with DHL (25). More recently, a 75% 3-year survival of patients with DPDL and DM treated with CHOP (26) and an actuarial survival of 60% at 4 years with disease-free survival of 87% at 3 years for similar patients treated with COPP or COPA (17) have been reported. The tendency of remission duration curves to plateau may indicate cure in some of these patients. Our 58% CR rate for 26 patients within the DPDL and DM subgroups treated with M-BACOD (table 2) and the observed 67% disease-free survival at 5 years in diffuse non-T-cell patients (fig 4) again suggest durable responses after M-BACOD therapy.

Prognostic factors which have been reported to adversely affect the results of non-Hodgkin's lymphoma therapy include age > 50–65 years (37,38), systemic symptoms (38,39), extranodal disease other than bone marrow and liver (40), and prior chemotherapy (41). In our series of 44 patients, the median age was 50 years (range, 18–75) and there was no significant difference in response rate to M-BACOD by age. All of our patients had systemic symptoms and 80% had bulk disease. Nine patients had extranodal disease outside the bone marrow or liver and responded no differently than other advanced-stage patients. Bone marrow involvement similarly did not influence either response or relapse rate. Finally, 11 patients in our series were previously treated; their 55% CR rate did not statistically differ from the 57% response rate in untreated patients. It therefore appears that the M-BACOD regimen can achieve durable CR in patients with features predictive of poor prognosis.

The toxicity of the M-BACOD treatment program can be severe, as evidenced by our two of 44 (4%) treatment-related deaths. The other observed toxic effects, ie, myelosuppression, and bleomycin-related pulmonary or methotrexate-related renal compromise, are not unlike those related to combination chemotherapy regimens employing these and similar agents. Of the therapies employed to treat advanced-stage indolent lymphomas, daily single alkylating agents and whole-body irradiation are clearly the least toxic, combination chemotherapy is intermediate, and combined modality therapy is associated with the greatest host toxicity (9). One must clearly balance the toxicity and potential therapeutic benefit to each regimen. In our selected patients with multiple poor prognostic factors and aggressive disease, we felt that a trial of aggressive combination chemotherapy (M-BACOD) was warranted.

The M-BACOD program is more active in patients with the unfavorable DH or DUL (non-Burkitt's) lymphomas (28) than in those with advanced nodular, DM, and DPDL subgroups of non-Hodgkin's lymphoma described in this report. Lymphomas of the former type are more rapidly growing and are therefore more responsive to high-dose methotrexate than the latter more slowly growing tumors. Even in the setting of advanced DHL, however, preliminary data indicate that moderate-dose methotrexate (200 mg/m^2) may have similar efficacy to higher doses (42). Nonetheless, the 57% CR rate coupled with the 64% 5-year survival and 43% disease-free survival of complete responders in our series suggests utility of M-BACOD therapy in selected patients with favorable and intermediate prognosis lymphomas. The 25% (11 of 44 patients) overall disease-free survival of our patients with multiple poor prognostic features (advanced stage, systemic symptoms, and bulk disease) confirms that they indeed have more aggressive clinical disease than their histological classification alone would predict. Longer follow-up will be required to determine whether intensive therapy with M-BACOD results in any cures in these patients with advanced low- and intermediate-grade histopathologic subgroups of non-Hodgkin's lymphomas.

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Treatment of Myeloid Blastic Crisis of Chronic Myelogenous Leukemia[1,2]

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Patients with myeloid blastic crisis of chronic myelogenous leukemia were treated by chemotherapy or by autologous hematopoietic reconstitution after aggressive chemotherapy. Chemotherapy alone failed to produce a second chronic phase. Autologous transplantation resulted in the establishment of a second chronic phase in two of ten patients treated with a four-drug regimen, while treatment with high-dose cytarabine with or without busulfan resulted in the establishment of a second chronic phase in three of six patients and the return of normal hematopoiesis in a fourth. Consolidation chemotherapy appeared to be beneficial. [Cancer Treat Rep 68:1351–1355, 1984]

Blastic crisis, the terminal phase of chronic myelocytic leukemia (CML), is uniformly fatal, with patients surviving only several months after diagnosis (1). Chemotherapy is generally ineffective, with only the lymphoid variety of blastic crisis exhibiting a measure of responsiveness to therapy (2–7). This paper represents a cooperative effort at defining the role of both chemotherapy and autologous hematopoietic reconstitution with cryopreserved chronic phase cells in the treatment of blastic crisis.

METHODS

Definitions of Diagnosis and Response

Blastic crisis was defined as the presence of ⩾ 30% myeloblasts and promyelocytes in the bone marrow and/or peripheral blood. Patients with lymphoid morphology or surface markers were not studied. All patients had cytogenetically documented Ph[1] CML.

Responses to therapy were classified into treatment failure types (8).

(*a*) *Drug-resistant failure*—persistence of blast cells in blood or marrow ⩾ 7 days after the end of chemotherapy or regrowth of blastic cells in a marrow which was rendered hypocellular (< 5% cellularity on biopsy or < 1+ aspirate cellularity on a scale of 1–4+) by therapy.

(*b*) *Early death*—patient died < 7 days after the end of chemotherapy.

(*c*) *Hypoplastic death*—patient died between 7 and 40 days after the end of therapy with a hypocellular marrow *[see (a) above]*.

(*d*) *Regeneration failure*—persistence of pancytopenia and significant marrow hypocellularity for > 40 days after the end of therapy.

(*e*) *Return to chronic phase of CML in marrow and peripheral blood.*

Chemotherapeutic Programs

Cytarabine, Doxorubicin, Prednisone, Vincristine, and High-Dose Cytarabine Regimen

Doxorubicin (30 mg/m²/day) was given on Days 1–3; vincristine (2 mg) on Day 1; prednisone (40 mg/m²) on Days 1–7; cytarabine (100 mg/m²/day), by continuous infusion on Days 1–4; and high-dose cytarabine (3 g/m²), every 12 hours on Days 5–7. If available, cryopreserved chronic phase cells were infused on Day 10 after the start of therapy.

Consolidation therapy for patients in second chronic

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phase is as follows: four courses of therapy, each administered when the platelet count rose to ⩾ 100,000/μl and when the granulocyte count rose to ⩾ 1000/μl after the previous course of therapy. Consolidation courses 1 and 3 are as follows: doxorubicin (20 mg/m²) on Days 1 and 2 and cytarabine (75 mg/m²) every 12 hours sc on Days 1-5. Consolidation courses 2 and 4 are as follows: cytarabine (3 g/m²) every 12 hours for six doses.

High-Dose Cytarabine With or Without Busulfan Regimen

Patients without cryopreserved cells received either a single or two 3-day courses of cytarabine at 3 g/m² every 12 hours separated by 1 week. Patients with cryopreserved cells received two 3-day courses of cytarabine separated by 1 week. If the marrow was still cellular 2 days after the end of cytarabine therapy, a 4-day course of busulfan at 4 mg/kg/day was administered. Cryopreserved chronic phase cells were infused 2 days after the end of therapy. Consolidation therapy was the same as that described above.

Collection and Preservation of Chronic Phase Cells

All patients except 1 were leukapheresed at the time of or shortly after the diagnosis of CML had been made. A single patient had chronic phase bone marrow cells collected and stored. Cells were stored in the vapor phase of liquid nitrogen for 1-3 years.

Cytarabine, Doxorubicin, Prednisone, Vincristine, and High-Dose Cytarabine (table 1)

This regimen produced severe marrow hypoplasia in 13 of 14 patients. Four patients received only the chemotherapy regimen and of these, three were hypoplastic failures and one was a regeneration failure.

Ten patients were infused with their own cryopreserved cells. A second chronic phase of CML was established in two patients. One patient received no further therapy and had recurrent crisis on Day 65. The other patient received only consolidation course 1 and remained in the second chronic phase for 1 year.

Three patients had early death and one patient failed to engraft. Four patients failed because of resistant leukemia.

Split-Course High-Dose Cytarabine Regimen (table 2)

Thirteen patients were treated, with seven patients receiving only chemotherapy and six patients being autotransplanted. Of the seven patients who received 3 days of cytarabine, six were drug-resistant failures and there was one early death. Three patients received two 3-day courses, with two early deaths and one regeneration failure. Two additional patients received only chemotherapy (high-dose cytarabine for 6 consecutive days), with one hypoplastic death and one relative drug-resistant failure.

Six patients were autotransplanted. Among the four patients who received high-dose cytarabine only as preparation, a second chronic phase was established in three. Two patients received high-dose cytarabine and busulfan. One died from liver failure, while the other failed to engraft.

Of the three patients in whom a second chronic phase was established, one patient did not receive consolidation chemotherapy due to viral hepatitis. Blastic crisis recurred on Day 60. The second patient received four courses of consolidation therapy and blastic crisis re-

TABLE 1.—Results for patients treated with cytarabine, doxorubicin, prednisone, vincristine, and high-dose cytarabine

Outcome	No. of patients	Comments	Survival (days)
		Chemotherapy only	
Hypoplastic death	3	—	21, 24, 27
Regeneration failure	1	Died aplastic with cerebral chloroma	54
		Chemotherapy * *+ autologous reconstitution*	
Drug-resistant failure	4	1 patient with persistent blastic crisis; 3 patients rendered hypocellular, recurrent blastic crisis on Days 32, 32, and 33, respectively	270, 90, 89, retransplanted (table 2)
Early death	3	2 septic deaths and 1 death due to myocardial infarction; on postmortem examination the latter patient had residual blastic crisis	12, 20, 22
Nonengraftment	1	Aplastic for 5 mos then had recurrence of blastic crisis	180
2nd chronic phase	2	1 patient engrafted on Day 26, had splenectomy, recurrent blastic crisis on Day 65;	84
		1 patient engrafted on Day 54, received consolidation course 1, and remained in 2nd chronic phase for 1 yr	502

* Median age in yrs (range): 50.5 (24-57). Median duration in mos of chronic phase prior to blastic transformation (range): 24 (2-90). Median No. of cells transplanted (range): 1.5×10^{10} (2.8×10^{8} to 4×10^{10}).

TABLE 2.—Results for patients treated with high-dose cytarabine with or without busulfan

Treatment	Outcome	No. of patients	Comments	Survival (days)
			Chemotherapy only	
3-day course of high-dose cytarabine	Drug-resistant failure	4	1 patient received 3 days of therapy and had persistent blastic crisis	51
			1 patient received a 2nd 3-day course because of persistent disease and was a regeneration failure	71
			In 1 patient a 3-day course rendered the marrow hypocellular; blastic crisis recurred on Day 17 and the patient died on Day 116	116
			In 1 patient a 3-day course of therapy rendered the marrow hypocellular; blastic crisis recurred on Day 24; patient received 2 subsequent 3-day courses separated by 1 week; crisis recurred after 34 days	158
	Extramedullary persistence	1	Splenomegaly persisted and the patient died	36
	Early death	1	Patient died on Day 4	4
	Death, failure type inevaluable	1	Received 2–3-day course of therapy and died; no bone marrow examinations	12
6-day course of high-dose cytarabine	Hypoplastic death	1		33
	Drug resistance	1	Marrow was rendered hypocellular, but blastic crisis recurred on Day 34	60
			Chemotherapy + autotransplantation *	
High-dose cytarabine only	2nd chronic phase	3	2nd chronic phase established on Days 33, 40, and 55, respectively; 1 patient * not consolidated due to hepatitis; recurrent blastic crisis on day 60; 1 patient received 4 consolidations and had recurrent blastic crisis at 1 yr; 1 patient received 1 consolidation, remained thrombocytopenic; still alive	134, 413, and 377+, respectively
	Septic death	1	Received cryopreserved chronic phase marrow cells	29
High-dose cytarabine	Regeneration failure	1	No engraftment; after 2 mos normal hematopoiesis returned with normal cytogenetics; alive and well	444+
	Died, liver failure	1	History compatible with viral hepatitis	10
High-dose cytarabine × 6 days + AMSA	Relative drug resistance	1	Recurrent blastic crisis on Day 54; treated on Protocol 1 (table 1) with same results	288

* Median age in yrs (range): 38(29–64). Median duration in mos of chronic phase prior to blastic crisis (range): 17(9–66). No. of cells transplanted: 1.5×10^{10} to 2.4×10^{10}.

curred in this patient 3 months after the end of consolidation therapy, 10 months after re-establishment of a second chronic phase.

The third patient received one course of consolidation therapy and remained thrombocytopenic (platelet count, $63.0 \times 10^3/\mu l$) for 3 months. Blastic crisis recurred at 5 months.

One patient was aplastic for 2 months following which *normal* hematopoiesis returned, with normal wbc count and differential, platelet count of 80,000/μl, and hematocrit of 39%. Two cytogenetic studies performed on bone marrow cells 7 and 9 months after treatment showed only normal metaphases.

DISCUSSION

The studies reported here were initiated after a retrospective review of patients with myeloid crisis treated with conventional-dose cytarabine alone or together with other agents demonstrated that a return to the chronic phase was produced in only one of 33 patients. Treatment failure was ascribable to persistent blastic crisis or early death. To obviate these problems we elected to explore autologous transplantation as a therapeutic adjunct to aggressive chemotherapy. As can be seen in the two studies described here, a return to the chronic phase occurred only in autotransplanted patients. In all, 13 patients re-

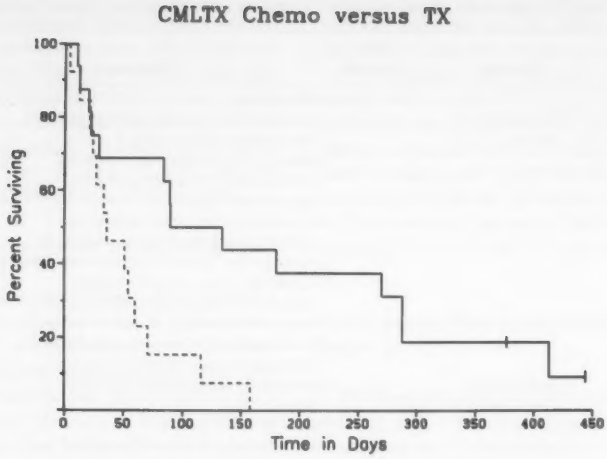

FIGURE 1.—Survival of transplanted and nontransplanted patients. ---- = treated with chemotherapy alone; ——— = treated with chemotherapy and autotransplantation.

ceived chemotherapy alone and 17 patients were transplanted. The transplanted patients survived significantly longer than those who were not transplanted (fig 1), with median survivals of 37 and 135 days, respectively. Median survival for patients who engrafted is 377+ days (fig 1).

Given that chemotherapy is ineffective and that most patients are too old to receive marrow allografts, autografting offers the best possibility of prolonging the survival of patients in myeloid crisis. A significant problem with this approach to therapy is the early recurrence of blastic crisis (9). An attempt to deal with this problem by increasing the aggressiveness of the preparative regimen resulted in an increase in mortality (10). Our approach, therefore, has been to attempt to increase the duration of the second chronic phase by administering consolidation chemotherapy once the chronic phase is re-established (11). This approach appears to be promising since blastic crisis recurred 2-3 months after transplantation in the two patients who did not receive consolidation therapy, while the second chronic phase lasted for 5, 11, and 12 months in those patients who received consolidation therapy. Autotransplantation during the accelerated phase or repeated autografts after ablative therapy might be even more beneficial.

In one patient normal hematopoiesis returned after autotransplantation appeared to have failed. A return of normal hematopoiesis has been reported after aggressive chemotherapy in autotransplanted (12,13) and in nontransplanted patients (14,15). The apparent complete suppression of the blastic phase clones by the high-dose cytarabine and busulfan regimen suggests that this combination may be superior to the high-dose cytarabine-alone regimen.

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Sensitive Radioimmunoassay for Cytarabine and Uracil Arabinoside in Plasma[1]

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A sensitive and specific radioimmunoassay for cytarabine (ara-C) or uracil arabinoside (ara-U) was established by using [^{3}H]ara-C and anti-ara-C antiserum or [^{3}H]ara-U and anti-ara-U antiserum. The antiserum was prepared by immunizing rabbits with ara-C or ara-U hapten conjugated to human albumin. In the present assay, as low as 2.5 ng/ml (0.01 μM) of ara-C or 5 ng/ml (0.02 μM) of ara-U in blood plasma samples could be detected. The cross-reaction of ara-C or ara-U structurally related compounds with each antiserum was so small that the nucleosides could be determined without any purification procedure. The plasma concentration of ara-C and ara-U in mice following oral administration of an antileukemic agent, cytosine arabinoside-5′-stearylphosphate (C18PCA) (100 mg/kg), or ara-C (48 mg/kg) was determined by using this method. In the case of C18PCA administration, ara-C was detectable in blood for at least 24 hours longer than in the case of ara-C administration. This method is suitable for the analysis of ara-C and ara-U, the metabolites of depot drugs of ara-C such as C18PCA. [Cancer Treat Rep 68:1357–1366, 1984]

It is widely known that the administration of cytarabine (ara-C), a clinically effective antileukemic drug, should be frequent to maintain the effective blood concentration, because the compound is rapidly converted to an inert metabolite, uracil arabinoside (ara-U), by the action of deaminase in a physiologic state (1). It is greatly beneficial, therefore, to modify ara-C to a deaminase-resistant form. We have been investigating the antitumor effect of a series of cytosine arabinoside-5′-alkyl or arylphosphate (2). Cytosine arabinoside-5′-stearylphosphate (C18PCA) (fig 1) was selected from the various ara-CMP esters as one of the most promising orally effective antileukemic drugs with a prolonged activity. Since it is important to investigate the absorption, distribution, metabolism, and excretion of C18PCA in humans and animals, we have tried to establish an assay method for the determination of ara-C and ara-U in biological samples such as tissue extracts and body fluids. Although several assay methods are now available to measure the arabinonucleosides, they are of limited use for the routine analysis of large numbers of biologic samples because of insufficient sensitivity, complexity of the procedure, or occasional interference by other substances (3–7).

This report describes a highly sensitive and specific assay technique for microdetermination of ara-C and ara-U on the basis of radioimmunoassay and its application to an analysis of plasma concentration of the arabinonucleosides in mice given C18PCA or ara-C.

MATERIALS AND METHODS

Reagents.—Sources of reagents were as follows: human serum albumin (crystallized), Miles Laboratories, Elkhart, IN; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, Nakarai Chemicals Ltd, Kyoto, Japan; [5-^{3}H]ara-C and aqueous counting scintillant (ACS II), Amersham International Ltd, United Kingdom; complete Freund's adjuvant, Iatoron Laboratories, Tokyo,

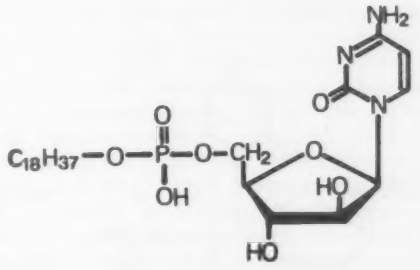

FIGURE 1.—Structure of cytosine arabinoside-5′-stearylphosphate (C18PCA).

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Japan; ara-C, ara-U, C18PCA, 2′-deoxycytidine, cytidine, 2′-deoxyuridine, ara-C-5′-monophosphate, 5′-CMP, and 5′-ATP, Yamasa Shoyu Company, Ltd, Choshi, Japan. Other reagents were of analytical grade from commercial sources.

Synthesis of succinyl ara-C and ara-U.—The succinyl nucleosides were prepared by the selective succinylation by means of general procedures (8–10). 2′-*O*-Succinyl ara-C was prepared as follows: ara-C-3′, 5′-diphosphate (3′, 5′-ara-CDP) was derivatized to N^4, 2′-disuccinyl-3′, 5′-ara-CDP by reaction with succinic anhydride in the mixture of formamide and tri-*n*-butylamine. N^4, 2′-disuccinyl-3′, 5′-ara-CDP was converted to 2′-succinyl-3′, 5′-ara-CDP by adjusting the pH to 2.0. The 2′-succinyl-3′, 5′-ara-CDP was incubated with acid phosphatase (EC 3.1.3.2) prepared by T. Fujishima in our laboratory to give 2′-*O*-succinyl ara-C, which was then purified in the usual way in a 48% yield. 3′-*O*-Succinyl ara-C was prepared as follows: 2, 2′-cyclocytidine-5′-monophosphate (5′-cyc-CMP) was succinylated at the 3′-*O* position by reaction with succinic anhydride in the mixture of formamide and tri-*n*-butylamine to make 3′-succinyl-5′-cyc-CMP. The 2-2′ bond thereof was cleaved by adjusting the pH to 10 to give 3′-succinyl-5′-ara-CMP, which was then converted to 3′-*O*-succinyl ara-C by the acid phosphatase treatment. The purified compound was obtained in a 42% yield. 5′-*O*-Succinyl ara-C was synthesized as follows: 2, 2′-cyclocytidine-3′-monophosphate (3′-cyc-CMP) was converted to 5′-succinyl-3′-cyc-CMP by reaction with succinic anhydride in the mixture mentioned above. By adjusting the pH to 10, 5′-succinyl-3′-cyc-CMP was transformed to 5′-succinyl-3′-ara-CMP. The acid phosphatase treatment of the intermediate gave 5′-*O*-succinyl ara-C. The yield of the purified product was 48%. Deaminating 2′, 3′, or 5′-*O*-succinyl ara-C with sodium nitrate gave 2′, 3′, or 5′-*O*-succinyl ara-U in a 48%–60% yield. The structure of the succinyl compounds shown in figure 2 was confirmed by UV, nuclear magnetic resonance, and elemental analyses.

Preparation of immunogen.—The immunogen was prepared by coupling the succinyl derivative thus prepared to human serum albumin. The succinyl nucleoside (200 mg) was mixed with 100 mg of human serum albumin and 100 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in 20 ml of 50 mM acetate buffer solution, pH 5.5. After incubation at 25°C for 20 hours in the dark, the mixture was dialyzed against running 0.9% NaCl solution, whose flow rate was 450 ml/hour, for 40 hours to separate the arabinoside-protein conjugate from the free nucleoside derivative. The ultraviolet spectrum of the dialyzed ara-C or ara-U conjugate had a maximum at 260 and 278 nm. From the difference between the extinction coefficient of succinyl-arabinoside-albumin and that of unconjugated albumin, the conjugate was estimated to contain 7.1 to 12.0 arabinoside residues per albumin molecule, as shown in table 1.

Immunization schedule.—Male rabbits of the Japanese white strain, weighing 2.0–2.6 kg, were immunized with an emulsion composed of equal parts of complete Freund's adjuvant and 0.9% NaCl solution containing succinyl-arabinoside-albumin. They were given injections of 0.2, 0.2, and 0.2 mg of the antigen in their backs at 10-day intervals. Fifty days later, the rabbits were immunized again in the same way. All the animals were bled from the carotid artery 10 days after the last injection, when the titer was expected to be maximal. The antisera were stored in small aliquots at -20°C.

Preparation of [^{3}H]ara-U.—Ethanol solution of [^{3}H] ara-C (500 μCi/250 μl) was concentrated in a nitrogen gas phase, and was dissolved in a mixture of sodium nitrite (450 mg), acetic acid (1 ml), and distilled water (1.5 ml) for deamination. The reaction mixture was allowed to stand overnight at room temperature; it was then filled with distilled water up to 30 ml. After passing through cation exchange resin (Diaion PK216, Mitsubishi Kasei-kogyo KK, Tokyo, Japan) and anion exchange resin (Amberlite IRA402, Organo KK, Tokyo, Japan) columns

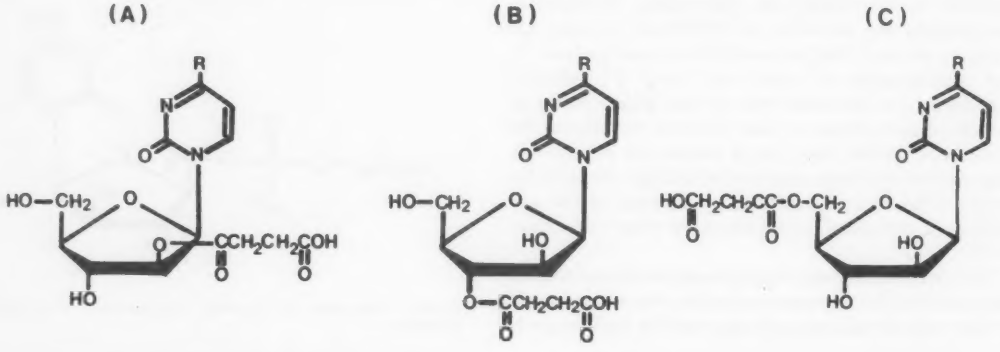

FIGURE 2.—Structure of succinyl ara-C and ara-U. R = $-NH_2$, ara-C; R = –OH, ara-U. (A) = 2′-*O*-succinyl derivative; (B) = 3′-*O*-succinyl derivative; and (C) = 5′-*O*-succinyl derivative.

TABLE 1.—Titer of antiserum raised against succinyl ara-C and ara-U conjugated to albumin*

Nucleoside	Succinylation site	Molar ratio (hapten/carrier)	Titer of antiserum, B/T (%), in rabbit No.— 1	2	3	4	5	6	7	8
Ara-C	2′-*O*	11.9	8.2	0.9	0.8	0.6	0.0	0.0	0.0	—
	3′-*O*	11.6	55.8	50.7	48.9	3.1	1.7	1.2	1.0	1.0
	5′-*O*	8.9	77.7	63.0	61.1	56.7	2.0	1.5	0.9	—
Ara-U	2′-*O*	7.1	NC †	NC	NC	NC	NC	NC	NC	NC
	3′-*O*	8.8	83.6	69.2	56.4	31.1	30.6	26.2	22.7	8.0
	5′-*O*	12.0	83.1	74.0	55.6	42.8	26.5	21.8	16.5	—

* Incubation of radioligand with antiserum was carried out in an ice-cold water bath for 1 hr. Antiserum was 200-fold diluted in the incubation medium. B/T(%) = (radioactivity bound with antiserum/total radioactivity) × 100.
†NC = not challenged.

(20 ml each of wet resin) for deionization, the solution was concentrated to dryness and was dissolved in 66% ethanol (500 μl). [^{3}H]ara-U was isolated by thin-layer chromatography on a cellulose F254 (E. Merck Japan) plate which was developed with a mixture of isopropanol, ethylacetate, distilled water, and triethylamine (20-20-10-0.05, vol/vol). The spot of ara-U was collected and extracted with distilled water (5 ml) overnight at room temperature. The solution of [^{3}H]ara-U was diluted with 0.3 M imidazole buffer for use (16,000–20,000 cpm in an amount ranging from 0.7 to 0.9 pmol).

Preparation of dextran-coated charcoal.—Dextran-coated charcoal was employed for adsorbing the unbound arabinoside. The dextran-coated charcoal was prepared by adding 500 mg of bovine serum albumin (Fraction No. V, Sigma Chemical Co, St. Louis, MO), 75 mg of dextran (molecular weight of 0.8–2.4 × 10^5), and 500 mg of Norit Extra to 100 ml of distilled water. The mixture was stored in a cold room before use.

Assay procedure.—The standard assay mixture consisted of 10 μl of a standard solution (ara-C or ara-U) or an unknown sample solution, 100 μl of the radioactive ligand solution (16,000–20,000 cpm/0.7–0.9 pmol), and 100 μl of the antiserum solution diluted with 50 mM sodium acetate buffer solution, pH 6.0, containing 0.3% of bovine serum albumin. The binding reaction between antigen and antibody was carried out in an ice-cold water bath for 1 hour. A cold solution of dextran-coated charcoal (0.5 ml) was added to the reaction mixture. After 15 to 20 minutes, charcoal was spun down, and 0.5 ml of the supernatant containing bound antigen was counted for radioactivity in a liquid scintillation counter.

Comparison between direct assay and assay after extraction.—Nonfast male BDF_1 mice, weighing 29–33 g, orally received C18PCA (100 mg/kg) or ara-C (48 mg/kg). After 1 or 3 hours, they were decapitated to bleed. $ZnSO_4$ was added to the heparinized blood at a final concentration of 10 mM. After centrifugation, HCl was added to the plasma at a final concentration of 0.1 N. A portion of the plasma was directly analyzed for its ara-C and ara-U content by the present assay method. Residual plasma was mixed with an equal volume of trichloroacetic acid (TCA) (10%), and the mixture was centrifuged. The acid in the deproteinized supernatant was removed by extraction with water-saturated ether. The water layer was analyzed for its nucleoside content as described in "Assay Procedure."

Preparation of plasma sample.—Whole-blood specimens (0.02 ml) withdrawn from the tail vein of nonfast male BDF_1 mice, weighing 21–25 g, which had received a drug were rapidly mixed with 0.02 ml of saline containing 20 mM $ZnSO_4$ and heparin. After centrifugation (2540 × *g*, 5 minutes), 0.2 N HCl (0.02 ml) was added to the supernatant (0.02 ml) as diluted plasma. Ten microliters of the mixture was directly submitted to the present radioimmunoassay for ara-C or ara-U as described in "Assay Procedure."

RESULTS

Relationship between succinylation site of hapten and the characteristics of antisera.—Male rabbits of the Japanese white strain (seven or eight for each group) immunized with different kinds of hapten-albumin conjugates had antibody titers as shown in table 1. When 3′-*O* or 5′-*O*-succinyl arabinoside of cytosine or uracil was used as the hapten, high-titer antisera were produced in three or four rabbits per group. The antisera were capable of binding > 40% of [^{3}H]ara-C or [^{3}H]ara-U (0.8 pmol) at a dilution of 1:200 after 1 hour of incubation. When 2′-*O*-succinyl ara-C was used, however, the high titer was not produced. Immunization with 2′-*O*-succinyl ara-U-albumin conjugate was not carried out. The specificity of ara-C antisera is shown in table 2. Among the structurally related compounds, arabinosylcytosine-5′-monophosphate (ara-CMP) was cross-reactive with the antisera employed, especially the antisera raised against 5′-*O*-succinyl ara-C hapten. One of them, C502, did not discriminate ara-CMP from ara-C at all. The most specific antiserum was C305, which was produced in one of eight rabbits immunized with 3′-*O*-succinyl ara-C albumin conjugate. The cross-reaction of structurally related compounds with

TABLE 2.—Cross-reactivity of various compounds with ara-C antiserum*

Compounds tested	5′-*O*-Succinyl ara-C				3′-*O*-Succinyl ara-C		
	C502†	C510	C513	C515	C301	C303	C305
Ara-C	100	100	100	100	100	100	100
2′-Deoxycytidine	—	0.036	0.105	0.037	0.593	0.023	0.005
Cytidine	0.533	0.017	0.038	0.016	0.273	0.010	0.015
Ara-U	0.114	0.364	0.286	0.520	0.003	0.865	0.003
2′-Deoxyuridine	—	0.001	0.006	0.005	0.003	0.002	0.003
5′-Ara-CMP	100	10.8	11.6	12.9	8.8	8.200	4.639
5′-CMP	—	0.001	0.006	0.005	0.003	0.002	0.003
C18PCA	8	0.520	0.585	0.285	0.273	0.200	0.075
5′-ATP	—	0.001	0.006	0.005	0.003	0.002	0.003

*The radioimmunoassay was performed as described in the Materials and Methods section. Values are expressed in "relative binding affinity," which was calculated as [ara-C]/[compound tested] × 100, where [ara-C] and [compound tested] are concentrations required to cause a 50% displacement of labeled ligand binding.

†Values = antiserum No.

ara-U antisera is shown in table 3. Ara-CMP was also the most cross-reactive with antisera raised against the 5′-*O*-modified antigen. Regarding all other compounds tested, the cross-reactivity was negligible. The radioimmunoassay for ara-C and ara-U performed thereafter was carried out by using the antisera, C305 and U343, respectively. If the plasma concentration of ara-CMP is relatively high compared with that of ara-C or ara-U, it is understood that ara-C or ara-U is estimated to be higher than the real value. To learn the plasma ara-CMP level 1 and 3 hours after C18PCA administration in BDF_1 mice, the acid extracts of mouse plasma samples were treated with an excess amount of phosphomonoesterase (EC 3.1.3.1.), which had been confirmed to convert ara-CMP to ara-C quantitatively, and ara-C levels before and after the treatment were determined by the present radioimmunoassay. The ara-C levels (mean ± SEM, nmol/ml plasma) before and after the treatment were 2.66 ± 0.21 and 2.54 ± 0.15 after 1 hour and 2.10 ± 0.26 and 2.16 ± 0.23 after 3 hours, respectively. Hence, the content of ara-CMP was assumed to be negligible in plasma. Thus, owing to the high specificity of the antiserum, ara-C and ara-U can be successfully assayed without any prior purification procedure.

Time for equilibration of antigen-antibody reaction.—As shown in figure 3, the antigen-antibody reaction reached nearly 100% equilibrium within 2 hours for each assay. The slope of the standard curve even after as short as 15 minutes of incubation was steep enough for the determination of ara-C or ara-U. The lowest limit of significant detection of each nucleoside was 0.2 pmol/tube.

Validity of the assay.—Biologic samples such as crude plasma may contain substances which interfere with the antibody-radioligand interaction. This potential problem was excluded by running the following test. The plasma arabinonucleoside concentration was determined either directly or indirectly (after TCA extraction) for the same mouse samples collected 1 or 3 hours after the administration of C18PCA or ara-C, as described in the Materials and Methods section. There was no significant difference between the results obtained directly and indirectly: the results (mean ± SEM, nmol/ml plasma from four mice) obtained directly and indirectly were 0.59 ± 0.04 and 0.60 ± 0.14 (ara-C 1 hour after C18PCA) and 4.80 ± 0.61 and 4.95 ± 0.68 (ara-U 1 hour after C18PCA), respectively. Thus, the extraction may not be required. In addition, perseverance of each antiserum employed against varying volume of crude plasma prepared from human beings or BDF_1 mice in an assay tube was investigated. As a result, the upper limit of plasma volume for the assay without interference was 20 μl for ara-C antiserum C305 and 10 μl for ara-U antiserum U343 (data not shown). As

TABLE 3.—Cross-reactivity of various compounds with ara-U antiserum*

Compounds tested	5′-*O*-Succinyl ara-U				3′-*O*-Succinyl ara-U		
	U508†	U509	U510	U513	U301	U343	U347
Ara-U	100	100	100	100	100	100	100
2′-Deoxyuridine	0.128	0.160	0.170	0.200	0.711	0.595	0.927
Uridine	0.028	0.144	0.273	0.040	0.119	0.040	0.159
Ara-C	0.016	0.118	0.038	0.020	0.005	0.008	0.006
2′-Deoxycytidine	0.002	0.002	0.002	0.003	0.003	0.003	0.006
5′-Ara-CMP	1.280	2.286	2.429	1.389	0.051	0.039	0.006
C18PCA	0.040	0.073	0.053	0.046	0.003	0.003	0.006
5′-ATP	0.002	0.002	0.002	0.003	0.003	0.003	0.006

*See asterisk in table 2 for details.

†Values = antiserum No.

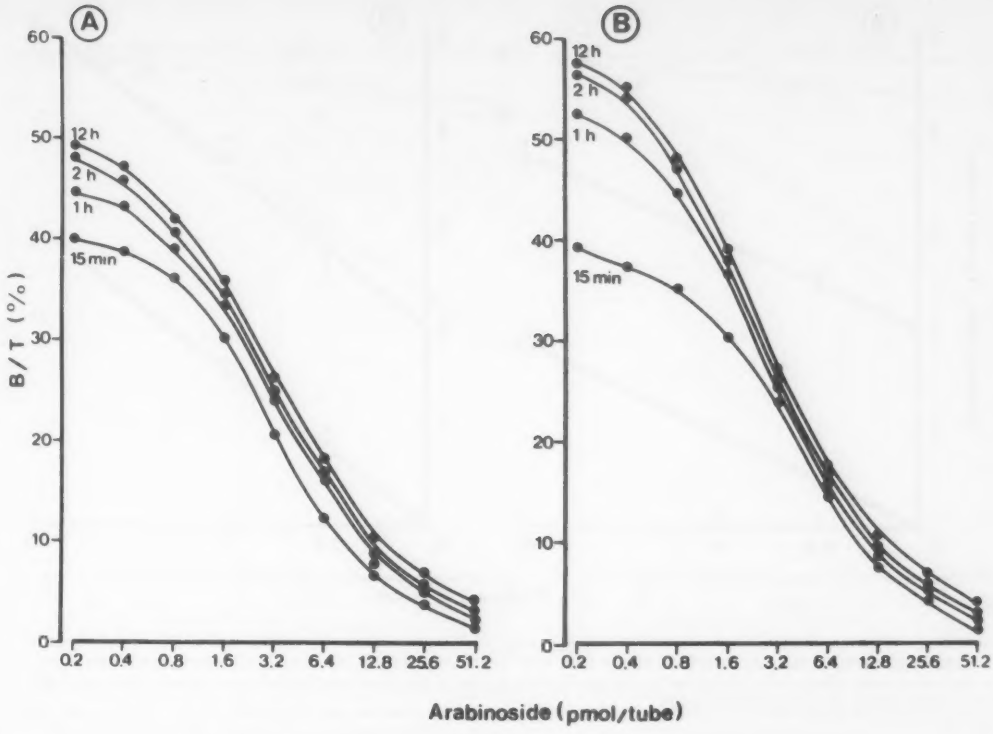

FIGURE 3.—Effect of incubation time on standard curves of ara-C (A) and ara-U (B).

shown in figure 4, the value of each arabinonucleoside obtained by the present assay method was proportional to the amount of mouse plasma per tube. In addition, 2 pmols of ara-C or ara-U added to the diluted plasma were quantitatively recovered. The reproducibility was studied at three levels of the arabinonucleosides. As shown in table 4, the between-assay coefficient of variance for each concentration was between 5.2% and 13.0% in the ara-C and ara-U assays. The intra-assay coefficient of variance for six aliquots from the same plasma analyzed in a single assay was < 10% for each concentration level. Both between-assay and intra-assay reproducibility seem satisfactory.

Spontaneous decrease in ara-C added to plasma or blood.—The concentration of ara-C and ara-U added to plasma or blood in vitro was determined as a function of time. As shown in figure 5, ara-C in plasma or blood decreased in a time-dependent manner. The decrease was greater in human materials than in mouse ones. Disappearance of ara-C from blood was protected by an addition of $ZnSO_4$ at a final concentration of 10 mM. A single addition of $ZnSO_4$ did not completely prevent the decrease in plasma ara-C. Further addition of HCl (0.1 N) was confirmed to be effective in maintaining the initial plasma level of ara-C. In contrast with the concentration of ara-C, the concentration of ara-U in plasma or blood did not change (data not shown). Human plasma ara-C was stoichiometrically converted to ara-U, probably due to the action of plasma cytidine deaminase (table 5).

Plasma concentration of ara-C and ara-U in BDF_1 mice after oral administration of C18PCA or ara-C.—The arabinonucleoside concentration in BDF_1 mouse plasma was periodically measured after oral administration of C18PCA or ara-C at a dose of 0.02 or 0.2 mmol/kg (10 mg or 100 mg/kg of C18PCA and 4.8 mg or 48 mg/kg of ara-C). As early as 15 minutes after the administration, ara-C was detected in the mouse plasma of each group as shown in figure 6. The peak of ara-C concentration was observed 30 minutes after ara-C administration or 60 minutes after C18PCA administration. The peak value of plasma concentration of ara-C derived from C18PCA was lower than that derived from ara-C. However, C18PCA (100 mg/kg) continued to supply ara-C to the blood for at least 24 hours, while ara-C completely disappeared from the blood 4 hours after ara-C administration. The plasma concentration of ara-U, whose peak was severalfold high-

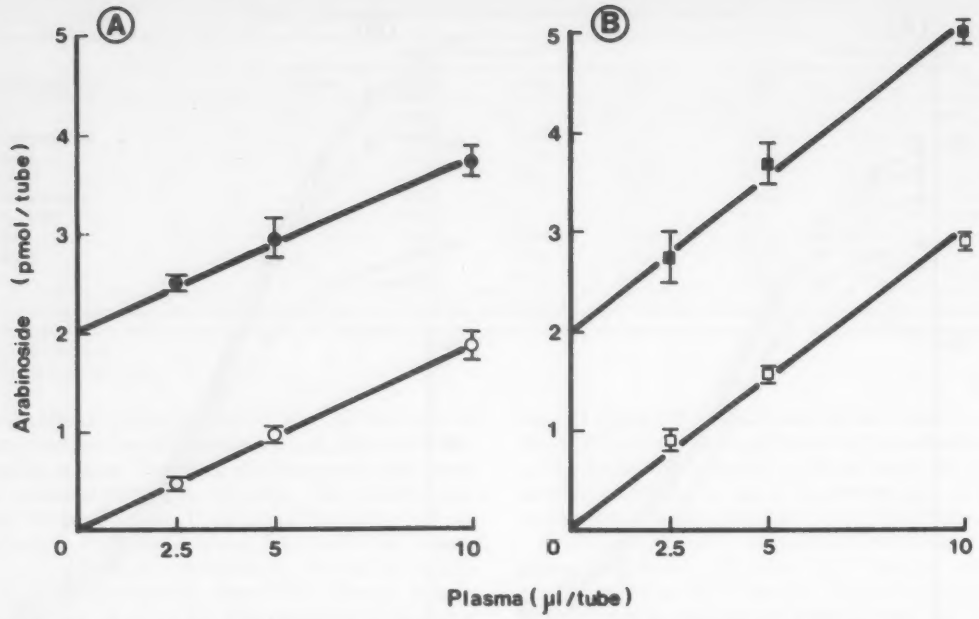

FIGURE 4.—Validation of the radioimmunoassay for ara-C (A) or ara-U (B). Plasma was obtained from BDF_1 mice which had orally received C18PCA (10 mg/kg) 30 mins ago. Various dilutions of the plasma were analyzed for ara-C (○) or ara-U (□) content. In addition, each diluted plasma was spiked with a constant known amount (2 pmols) of ara-C or ara-U and was measured again for ara-C (●) or ara-U (■). Each point represents mean ± SD in triplicate.

er than that of ara-C in each group, could be detected by the radioimmunoassay for at least 24 hours after administration of C18PCA or ara-C (fig 7).

DISCUSSION

The production of a suitable antiserum is the most important step for developing an immunoassay system, because it is the antibody that determines sensitivity and specificity of the assay. Ara-C and ara-U, whose molecular weight is < 250, had to be conjugated to albumin to stimulate an immunogenic response in the rabbit. For direct conjugation, the molecule of arabinonucleosides was selectively succinylated at 2′-*O*-, 3′-*O*-, or 5′-*O*-position of the arabinose ring to make a bridge of four carbon atoms in length which had been indicated to increase the immunogenicity of the hapten (11). In the present study, 2′-*O*-succinyl ara-C-albumin conjugate gave no suitable antiserum, in spite of the sufficient molecular ratio of 2′-*O*-succinyl ara-C to albumin, 11.9. On the other hand, 3′-*O*- or 5′-*O*-substituted ara-C-albumin conjugate with the ratio of 11.6 or 8.9 successfully raised antibody activities (table 1). In addition, the ratio of around 10 had been proved to be effective in making the useful antisera against cyclic AMP, cyclic GMP, cyclic CMP, and adenosine (12-14). Since antibodies are usually directed against the exposed part of the molecule, 2′-*O*-succinyl ara-C moiety may be sterically hindered by the large carrier molecule. As shown in tables 1-3, not only the avidity discussed above but also the specificity was dependent

TABLE 4.—Between-assay reproducibility*

	Concentration in control samples†					
	Ara-C			Ara-U		
	Low	Middle	High	Low	Middle	High
Mean (pmols/assay tube)	0.68	3.82	18.32	0.46	1.83	16.75
Standard deviation	0.08	0.20	1.82	0.06	0.17	1.86
Coefficient of variance (%)	11.8	5.2	9.9	13.0	9.3	11.1

* The No. of assays was 6, performed by 2 investigators.
†Control samples were prepared from human plasma.

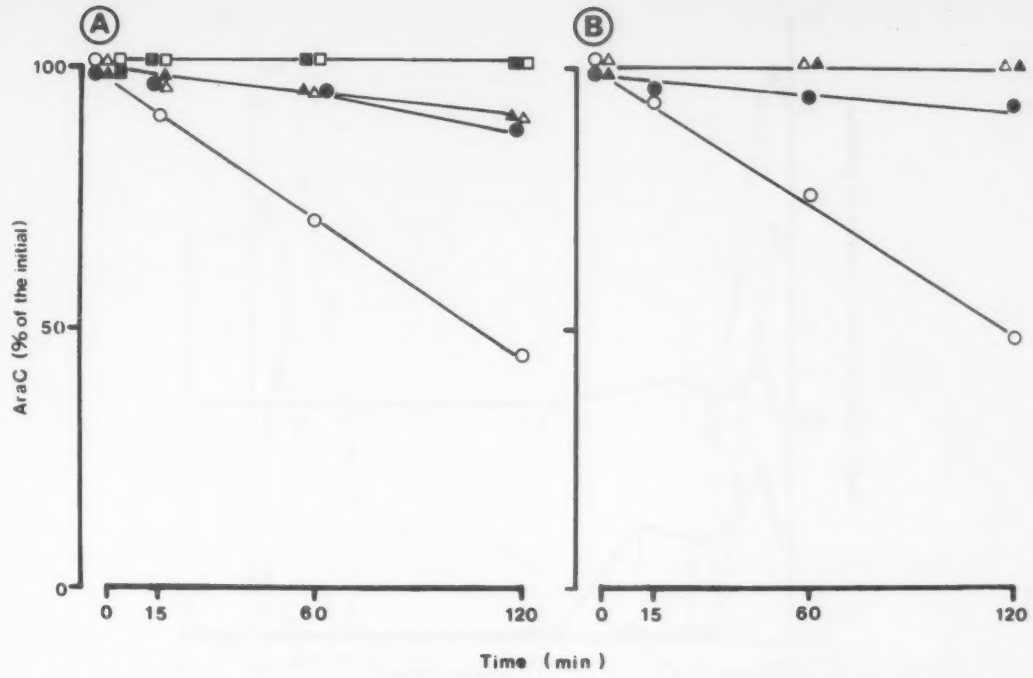

FIGURE 5.—Spontaneous decrease in ara-C concentration in plasma and blood and its inhibition. Blood was obtained from the cubitus vein of a human or by decapitation of BDF_1 mice. A portion of the heparinized blood was immediately centrifuged, and ara-C was added to the resultant plasma at a final concentration of 5000 pmols/ml. The mixture was stored at 37°C as shown on the abscissa of (A). Residual whole blood, to which ara-C had been added at a final concentration of 5000 pmols/ml, was stored at 37°C for the periods indicated on the abscissa of (B); at the indicated intervals, a fraction was withdrawn for separation of the plasma and treated with TCA and water-saturated ether as shown in the Materials and Methods section. Plasma concentration of ara-C measured by the radioimmunoassay is plotted against time of storage: ○, ● = without any addition; △, ▲ = with 10 mM $ZnSO_4$; and □, ■ = with 10 mM $ZnSO_4$ and 0.1 N HCl. Open symbols = human materials; solid symbols = mouse materials.

on the binding site of the hapten-protein conjugate. The conjugate containing 5′-*O*-succinyl arabinonucleoside as a hapten produced antisera cross-reactive with ara-CMP, which is regarded as one of 5′-*O*-modified ara-C. A similar result of the usage of 5′-*O*-succinyl ara-C and 5′-ara-CMP was previously reported by Okabayashi et al (4) and by Piall et al (7), respectively. In the present study, the strategy using the 3′-*O*-modified hapten proved to be superior in eliciting avid and specific antisera to that using the 2′-*O* or 5′-*O*-modified one. The assay sensitivity, as well as the avidity and the specificity, varied from antibody to antibody. There was no significant relation between the modification site of hapten and the sensitivity (data not shown).

In general, the assay becomes more sensitive as the specific radioactivity of a labeled antigen rises. Okabayashi et al (4) reported that the lowest limit of statistical detection of ara-C for their radioimmunoassay was 0.7 pmol by using [^{3}H]ara-C (2 pmols/tube) whose specific radioactivity was 8.19 Ci/mmol. In the present radioimmunoas-

TABLE 5.—Stoichiometry of ara-C deamination in human plasma *

Incubation time (mins)	Ara-C measured	Decrease in ara-C	Ara-U measured
0	5000	0	0
15	4282	718	677
60	3693	1307	1280
120	2342	2658	2704

* Human plasma supplemented with ara-C at a final concentration of 5000 pmols/ml was allowed to stand at 37°C, and at the indicated intervals, a fraction was withdrawn for measurement of the ara-C and ara-U content by the present radioimmunoassay. Values = pmols/ml of plasma.

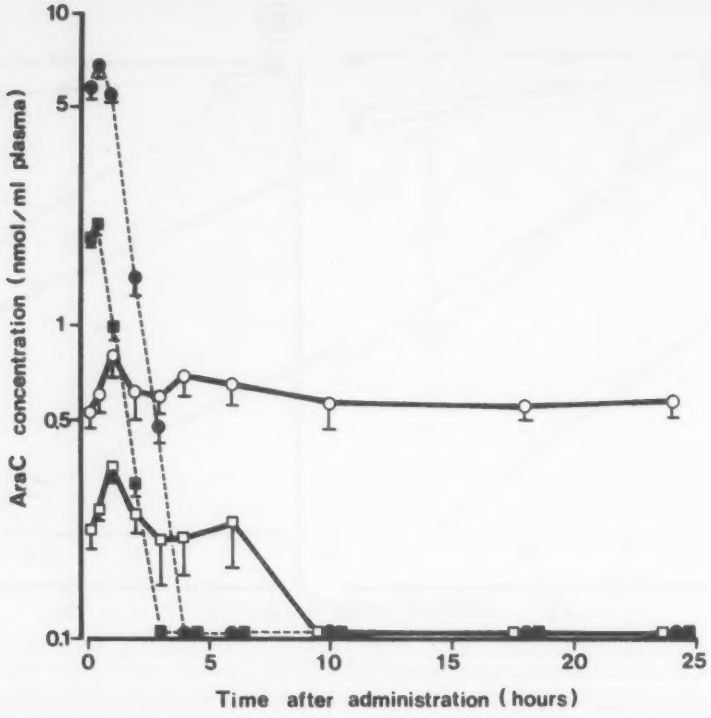

FIGURE 6.—Plasma ara-C concentration of BDF_1 mice after oral administration of C18PCA or ara-C. Preparation of plasma samples and determination of ara-C were performed as shown in the Materials and Methods section. Mean ± SEM for 3 mice is plotted as a symbol with a vertical line attached: ———○——— = C18PCA (100 mg/kg); ———□——— = C18PCA (10 mg/kg); ---●--- = ara-C (48 mg/kg); and ---■--- = ara-C (4.8 mg/kg).

say, the use of [^{3}H]ara-C or [^{3}H]ara-U in an amount < 1 pmol/tube with higher specific radioactivity (17.89 Ci/mmol) provided a more sensitive assay by which as little as 0.2 pmol/tube of the arabinonucleoside could be detected (fig 3). Furthermore, the upper limit of plasma volume for the assay without interference was 20 μl for ara-C antibody and 10 μl for ara-U antibody. From the above observations, it is concluded that the present assay could be used for the determination of ara-C and ara-U as low as 2.5 ng/ml (0.01 μM) and 5 ng/ml (0.02 μM) in crude plasma samples, respectively. Piall et al (7) previously developed such an ara-C radioimmunoassay that real sensitivity was almost equivalent to ours by using [^{3}H]ara-C in an amount of 0.1 pmol whose specific radioactivity was 15 Ci/mmol. However, the chemical amount of [^{3}H]ara-C used seems to be too small to give a sufficiently high count and to cover a long assay range. Actually, their assay range was 0.2–8 pmols/tube, while ours was 0.2–51.2 pmols/tube.

The good agreement between the plasma concentration of the arabinonucleoside in mice obtained with and without TCA treatment suggests that the circulating arabinonucleoside is not substantially included in the blood proteins. The validity of the present radioimmunoassay for measuring the nucleoside in crude plasma was demonstrated by the dilution and recovery test (fig 4). Accordingly, no extraction procedure of the nucleoside in crude plasma was required in the present assay system, while Okabayashi et al (4) previously reported the necessity of an ethanol treatment of plasma samples prior to ara-C determination to lessen interfering substances in crude plasma.

Ara-C in the plasma was stoichiometrically converted to ara-U by the action of cytidine deaminase (table 5). Therefore, prevention of a decrease in ara-C and an increase in ara-U was indispensable for the accurate and precise determination of both of them in such biologic fluids. As shown in figure 5B, the enzyme action in whole blood was perfectly inhibited by an addition of $ZnSO_4$ (10 mM). This result is well supported by the previous report of Tomchic et al (15) regarding mouse kidney cytidine deaminase which reacted to cytarabine and was inhibited by divalent metal ions such as Zn^{2+}, Cu^{2+}, etc. On the other hand, the maintenance of the initial level of plasma

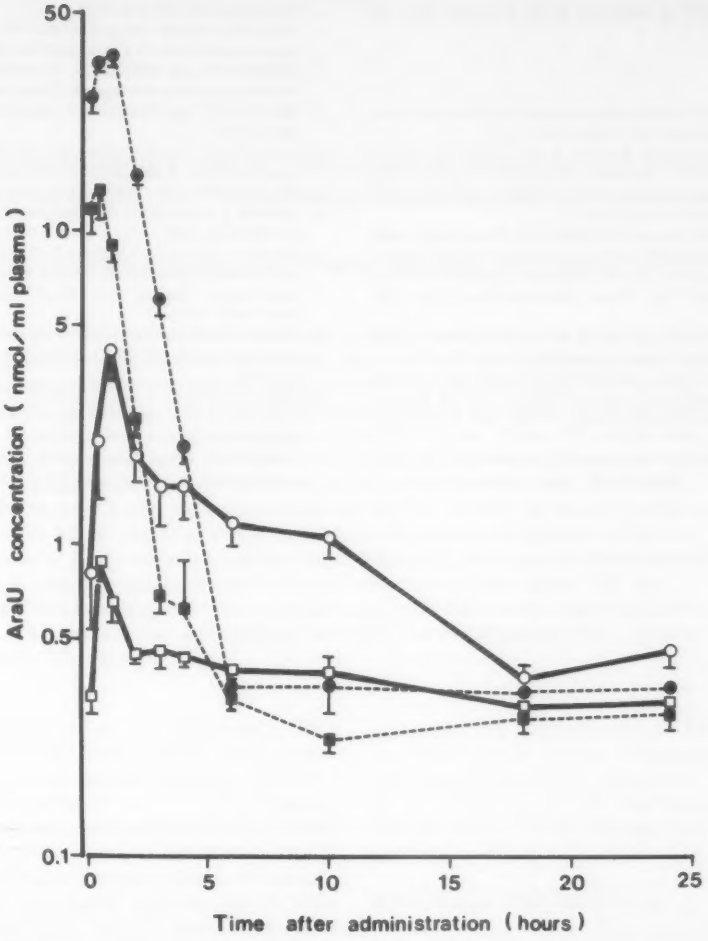

FIGURE 7.—Plasma ara-U concentration of BDF_1 mice after oral administration of C18PCA or ara-C. Preparation of plasma samples and determination of ara-U were performed as shown in the Materials and Methods section. *See* fig 6 for definitions of symbols.

ara-C required a combined addition of HCl (0.1 N) with $ZnSO_4$ (10 mM), as shown in figure 5A, which suggests the enzyme activity might be associated with blood cell components. However, the detailed mechanism remains to be investigated. Thus, the treatment with Zn^{2+} and HCl made it possible to obtain reliable values for the arabinonucleosides in the circulating blood.

The applicability of the present assay method was demonstrated in determining the concentration of ara-C and ara-U in the plasma of BDF_1 mice given C18PCA or ara-C (figs 6, 7). The sensitivity was so high that only 20 μl of the whole blood was sufficient for assays of ara-C and ara-U in plasma. Accordingly, the periodical change of arabinonucleoside concentration in the bloodstream could be followed in individual small animals such as mice by repeated withdrawal of blood specimens from the tip of the tail. By using the present radioimmunoassay, it was revealed that the plasma concentration of ara-C was kept much longer in mice which orally received C18PCA than in those which received ara-C (fig 6). Such prolonged existence of plasma ara-C from C18PCA could fully explain the potential effectiveness of C18PCA as an antileukemic agent, since the response to therapeutic agents was often more dependent on the blood concentration of the drug than on the administered dosage.

Thus, the present sensitive, simple, and reproducible method seems to be suitable, especially for the determination of plasma ara-C and ara-U, which are the major

metabolites of C18PCA inferred to be a depot form of ara-C.

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"Two-Route Chemotherapy" Using High-Dose Ip Cisplatin and Iv Sodium Thiosulfate, Its Antidote, for Peritoneally Disseminated Cancer in Mice[1,2]

Yukihide Iwamoto, Toyokazu Kawano, Jiro Uozumi, Ken Aoki, and Tsuneo Baba[3,4,*]

We studied the effects of "two-route chemotherapy (TRC)" using cisplatin given ip and its antidote, sodium thiosulfate (STS), given iv on mice bearing peritoneally disseminated cancer. Initially, a pharmacokinetic analysis of cisplatin given ip and STS given iv or sc was made. The plasma concentrations of non-protein-bound and total platinum increased rapidly to a maximum at 10 mins after ip administration of cisplatin alone. When STS alone was given sc, the peak level in the plasma was much lower than that given iv. The superiority of the iv route for STS administration was observed in the lethal toxicity tests. The iv administration of STS to mice 1 min after cisplatin given ip was the best protocol to protect against the lethal toxicity of cisplatin. The protective conditions were applied to TRC in which STS was given iv 1 min after ip cisplatin to mice bearing peritoneally disseminated cancer. Significantly superior antitumor effects were observed in mice given TRC by evaluating survival time as compared with mice given a single treatment with cisplatin at an equitoxic level. The nephrotoxic and hematotoxic effects were slight in TRC. [Cancer Treat Rep 68:1367–1373, 1984]

The local administration of an anticancer drug is often superior to systemic administration, since a high concentration of the drug can be maintained at the tumor site (1,2). Although the superior effects of ip administration of a drug for intraperitoneal tumors have been reported (3–5), the dose to be given is still limited since there are side effects once the drug enters the systemic circulation.

We reported the remarkable effectiveness of "two-route chemotherapy (TRC)" in the case of rat liver tumors, using an anticancer agent, mechlorethamine *N*-oxide (HN2-O), and its antidote, cysteine (6). HN2-O and cysteine were given via the hepatic artery and tail vein, respectively. In more recent studies (7–9), we selected a combination of cisplatin and sodium thiosulfate (STS) to treat liver, bladder, and peritoneally disseminated tumors in rats, since cisplatin possesses potent antitumor activities against a variety of experimental and human tumors, and STS has recently been reported to inactivate the toxicity of cisplatin (10,11). STS may safely be given in high doses since the toxicity is low in both humans and experimental animals (11,12).

We now report the results of pharmacokinetics of cisplatin and STS in plasma and of their combined effects on lethal toxicity in mice. When treating mice bearing peritoneal dissemination of a cancer, TRC using a combination of cisplatin and STS was found to be significantly superior to the single treatment with cisplatin, at an equitoxic level.

MATERIALS AND METHODS

Animals.—ddY female mice weighing 22–27 g at 6 weeks of age were used for pharmacokinetic and in vivo toxicity tests. DDD female mice weighing 19–25 g at 6–7 weeks of age were used for chemotherapy experiments. The DDD and ddY mice, obtained from the Animal Center of Kyushu University and from Kyudo Co, Ltd, Fukuoka, Japan, respectively, were maintained on a conventional diet and water ad libitum.

Chemicals.—Cisplatin was kindly provided by Nippon Kayaku Co, Ltd, Tokyo, Japan, and STS was obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Cisplatin and STS were each dissolved in 0.9% NaCl solution immediately before use. In all experiments described

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below, the volume of solution given to mice per 25 g of body weight was: 3 ml for cisplatin and 1 and 2 ml for 1750 and 3500 mg/kg of STS, respectively. For control mice given cisplatin alone, the same volume of saline as that of STS solution was administered. The infusion times of iv STS or iv saline via tail vein were 1.5 and 5 mins in the case of 1 and 2 ml/25 g of body weight, respectively, in all experiments. Under these conditions, no serious cardiovascular or hemodynamic effects occurred.

Pharmacokinetics of cisplatin and STS.—For pharmacokinetic studies of cisplatin, mice (five animals per group) were injected ip with 10 mg/kg of cisplatin and killed 1 min to 24 hrs after cisplatin administration. For assay of non-protein-bound platinum [which provides a good indication of free circulating cisplatin levels (13)], the protein was removed from plasma of each mouse by centrifugation for 10 mins at 1000 × *g* through CF-25 conical filters (Amicon Corporation, Danvers, MA). The concentrations of total and non-protein-bound platinum were estimated by flameless atomic absorption spectrophotometry (Atomic Absorption Spectro-photometer, Type 180-70, Hitachi, Co, Ltd, Tokyo, Japan) (14). For the pharmacokinetic study of STS, mice (six animals per group) were given 1750 mg of STS/kg of body weight iv or sc and killed 1–120 mins after this administration. The plasma was separated and the concentration of thiosulfate was estimated by the direct titration of thiosulfate with iodine, as described by Brun (15).

Lethal toxicity tests.—Ability of iv STS to protect against ip cisplatin-induced lethal toxicity was determined under the following conditions: to determine the best timing of iv STS, a fixed dose of 1750 mg/kg of STS was given iv at various times before and after ip administration of cisplatin (40, 50, and 60 mg/kg); and to assess the superior route of STS, various doses of cisplatin were given ip and a fixed dose of 1750 mg/kg of STS was given iv or sc at the most effective timing for protection against lethal toxicity of cisplatin. The LD50 value for each route of STS was determined.

Chemotherapy experiments.—A squamous cell carcinoma (MCUCa) induced in the uteri of DDD mice with 20-methyl-cholanthrene has been maintained by serial transplantation in the hind limb of syngeneic DDD mice. The tumor was excised, minced, and suspended in phosphate-buffered saline and passed through a metal sieve to obtain a single-cell suspension. After a trypan blue exclusion test, groups of ten mice were injected ip with 10^6 viable cells. Treatments were given on Day 3 or on Day 5 after the inoculation. In the present study, the cisplatin dose ratio for the group of TRC using cisplatin plus STS to the group of cisplatin treatment without STS was fixed at 5.6:1 to give an equitoxic level in both groups. In the group given cisplatin treatment alone, saline was given instead of STS solution. We did not give cisplatin in doses > 8.8 mg/kg in a single treatment or > 49.3 mg/kg in TRC because severe side effects were observed in the preliminary experiments. The stage of peritoneally disseminated cancer was assessed histopathologically at the start of therapy in a few mice. On Day 3 after the inoculation, small tumor nodules were found on the surface of the liver, pancreas, omentum, spleen, and ovary; on Day 5, the tumor nodules were enlarged, but never infiltrated to the parenchyma of abdominal organs.

Side effects.—The tumor-bearing mice were weighed on various days after the treatments. To determine the possible nephrotoxicity and hematologic disorders, mice without tumor were killed on various days after administration of the drugs. Serum BUN levels were measured by the urease technique (16) as an index of nephrotoxicity and leukocytes were measured as an index of hematologic toxicity.

RESULTS

Figure 1 indicates the plasma platinum elimination curve after ip administration of cisplatin. Both the total and non-protein-bound platinum concentrations increased rapidly up to 10 mins, and thereafter non-protein-bound platinum concentration declined exponentially with time while total platinum concentration declined

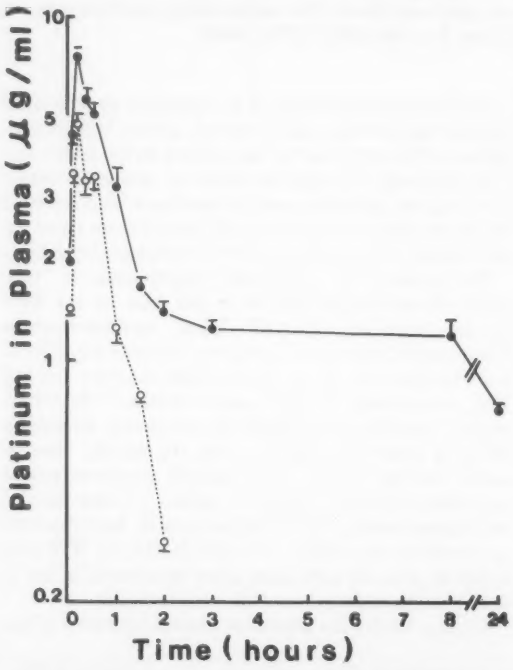

FIGURE 1.—Plasma levels of total and non-protein-bound platinum following ip administration of 10 mg/kg of cisplatin. Bar represents SE of the mean (n = 5). ● = total platinum; ○ = non-protein-bound platinum.

with biphasic pattern. The mean peak plasma concentrations were 4.96 μg/ml ± 0.43 (SEM) in non-protein-bound platinum and 7.61 μg/ml ± 0.69 (SEM) in total platinum. The half-lives calculated from platinum plasma concentration-time profile were approximately 28 mins in non-protein-bound platinum and approximately 33 mins (initial half-life) and 25 hrs (terminal half-life) in total platinum. Detectable levels of non-protein-bound platinum could be observed for only 2 hrs after cisplatin ip administration.

Figure 2 shows the plasma STS elimination curve after sc or iv administration of STS. The mean peak concentrations of STS were 4.73 mg/ml ± 0.14 (SEM) and 1.10 mg/ml ± 0.05 (SEM) in the groups of iv and sc administration, respectively. The half-lives of STS, calculated from its plasma concentration-time profile, were 10 and 27 mins, respectively, in the iv and sc groups.

Figure 3 shows the effect of timing of STS iv administration on the protection against ip cisplatin-induced lethal toxicity. At a dose of 40 mg/kg of cisplatin, all mice were protected against lethal toxicity by STS administration during 5 mins before cisplatin to 10 mins after this drug. At a dose of 50 mg/kg of cisplatin, STS was effective with a rather narrow range of time extending from 2 mins before and after cisplatin. In the case of 60 mg/kg of cisplatin plus STS, the smallest decrease in body weight of the survivors was obtained when STS was given 1 min after cisplatin (data not shown), although nearly a plateau level in survival was seen within a range of 0–5 mins (fig 3). Therefore, we chose the timing of iv STS 1 min after cisplatin. However, the most effective timing of STS sc administration for the protection against the cisplatin-induced lethal toxicity was 1 min before cisplatin ip administration (9).

In the next experiments, the timing of sc and iv administration of 1750 mg/kg of STS was fixed, respectively, at 1 min before and 1 min after cisplatin administration and various doses of cisplatin were given ip to mice. As shown in figure 4, the LD50 value of cisplatin was 55 mg/kg when STS was given sc, whereas the value was increased up to 64 mg/kg when STS was given iv. Difference in survival rates between groups of iv STS and sc STS was significant in the Mantel-Haenszel test ($P < 0.01$), indicating that the iv route for STS administration appeared to be superior to the sc route for the protection against cisplatin lethal toxicity. As shown in figure 5, when the dose of STS iv was increased up to 3500 mg/kg, the LD50 value increased up to 124 mg/kg compared with the control value of about 22 mg/kg. The cisplatin dose ratio for the group of TRC using cisplatin plus STS to the group of cisplatin treatment without STS was 5.6:1. A single dose of STS at 3500 mg/kg was selected as an optimal dose, since we confirmed in preliminary experiments that a single administration of STS not exceeding this dose was safe and did not lead to unphysiological conditions. Although an additional dose of 1750 mg/kg of STS iv was given 20 mins after the first 3500-mg/kg STS iv administration, the LD50 value did not increase (data not shown). The elimination curve of ip cisplatin and iv or sc STS and the LD50 value of ip cisplatin in ddY mice were similar to those in DDD mice, although a small number of the latter was tested.

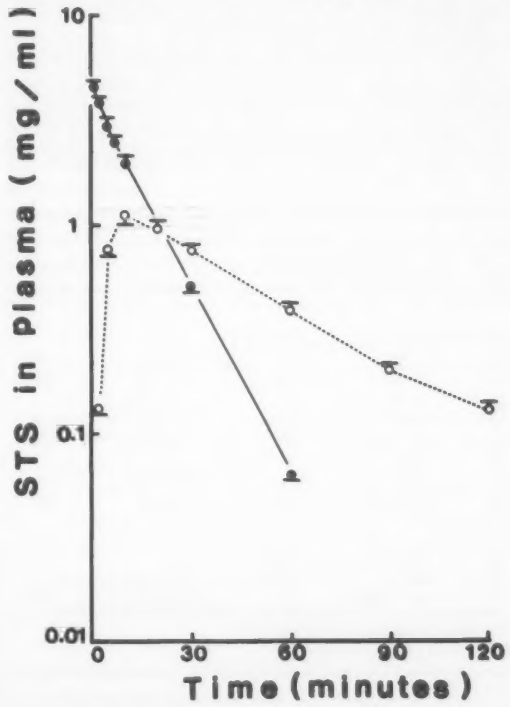

FIGURE 2.—Plasma levels of STS following iv and sc administration of 1750 mg/kg of STS. Bar represents SE of the mean ($n = 6$). ○ = sc administration; ● = iv administration.

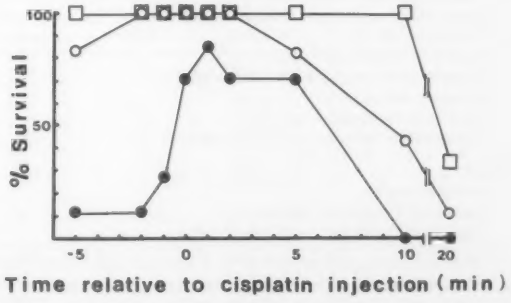

FIGURE 3.—Effect of timing of STS iv administration on the lethal toxicity of cisplatin given ip to mice. □ = cisplatin (40 mg/kg) + STS (1750 mg/kg); ○ = cisplatin (50 mg/kg) + STS (1750 mg/kg); ● = cisplatin (60 mg/kg) + STS (1750 mg/kg). Each point = % survival of 7 mice 30 days after treatments.

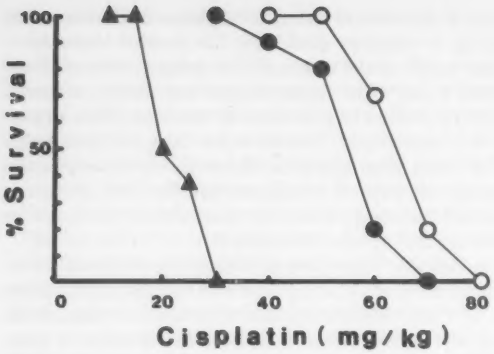

FIGURE 4.—Toxicity of cisplatin ip in mice with or without 1750 mg/kg of STS sc or iv. ○= cisplatin + STS iv; ● = cisplatin + STS sc; ▲ = cisplatin + saline iv. Each point = % survival of 10 mice 30 days after treatments.

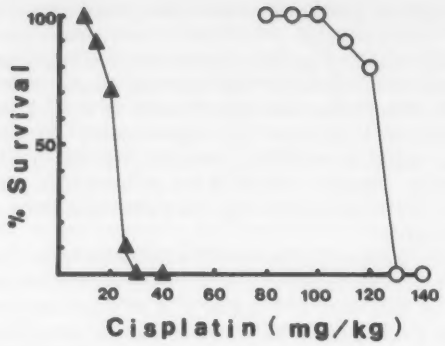

FIGURE 5.—Toxicity of cisplatin ip in mice with or without 3500 mg/kg of STS iv. ○ = cisplatin + STS iv; ▲ = cisplatin + saline iv. Each point = % survival of 10 mice 30 days after treatments.

On the basis of the results of lethal toxicity tests using ddY mice described above, chemotherapy experiments on DDD mice bearing peritoneally disseminated cancer were carried out. In TRC, mice were given 3500 mg/kg of STS iv (infusion time, 5 mins) 1 min after cisplatin ip administration. In the control group given single cisplatin treatment, the same volume of saline as that of STS solution was administered iv. The dose ratio of cisplatin was fixed at 5.6:1 for the group of TRC to the group of single cisplatin treatment so as to maintain the toxicity at the same level. The therapeutic effects were evaluated by survival time after the tumor inoculation. At autopsy, a large volume of bloody ascites and infiltration of tumor nodules into the abdominal organs were evident in all groups. The results of treatments are summarized in table 1. Increase in lifespan in the group of TRC was significantly greater than that in the control group without STS, as compared at each equitoxic level, and two of ten mice given TRC with 49.3 mg/kg of cisplatin survived > 250 days after the tumor inoculation.

Both of the groups of TRC and the single treatment of cisplatin showed a similar loss of body weight with a minimum level up to 3 days after the treatments, but the body weight began to increase and returned to the initial level 7 days after the treatments. On each day after the

TABLE 1.—Effect of various cisplatin treatments on survival time of mice bearing peritoneally disseminated cancer

Treatment*	No. of mice	Survival time (days)		P value†	% ILS‡
		Range	Median		
Day 3					
Untreated control	10	10-12	10		
Cisplatin (2.2 mg/kg) ip + saline iv§	10	11-18	14	< 0.05 (*a*)	40
Cisplatin (12.3 mg/kg) ip + STS (3500 mg/kg) iv§	10	18-29	25	< 0.05 (*b*)	150
Untreated control	10	10-13	11		
Cisplatin (4.4 mg/kg) ip + saline iv	10	12-36	23	< 0.05 (*c*)	109
Cisplatin (24.6 mg/kg) ip + STS (3500 mg/kg) iv	10	21-> 80¶	31	< 0.05 (*d*)	182
Untreated control	10	10-12	10		
Cisplatin (8.8 mg/kg) ip + saline iv	10	10-44	28	< 0.05 (*e*)	180
Cisplatin (49.3 mg/kg) ip + STS (3500 mg/kg) iv	10	22-> 80**	39	< 0.05 (*f*)	290
Day 5					
Untreated control	10	14-18	15.5		
Cisplatin (8.8 mg/kg) ip + saline iv	10	14-33	19.5	< 0.05 (*g*)	26
Cisplatin (49.3 mg/kg) ip + STS (3500 mg/kg) iv	10	19-> 250††	35	< 0.05 (*h*)	126

*Treatments were given on Day 3 or Day 5 after inoculation of 10^6 viable MCUCa cells into the peritoneal cavity.

†Probability was calculated by Mann-Whitney test. *P* values for (*a*) vs (*b*), (*c*) vs (*d*), (*e*) vs (*f*), and (*g*) vs (*h*): <0.05.

‡% increase in lifespan was calculated according to the equation: % ILS = 100(t/c - 1), where t is the median survival time of the treatment group and c is the median survival time of the control group.

§STS or saline was given to mice via the tail vein in a volume of 2 ml/25 g, with an infusion time of 5 mins.

¶At an autopsy of 1 surviving mouse on Day 80, ascites and tumor were not found.

**At an autopsy of 2 surviving mice on Day 80, ascites and tumor were not found.

††2 mice survived > 250 days after the tumor inoculation.

treatments, difference in the loss of body weight between the groups of TRC and the single treatment of cisplatin was not statistically significant (Student's t test; $P > 0.05$).

BUN levels after chemotherapy in mice without tumor were measured as an index of nephrotoxicity, a major dose-limiting factor of cisplatin (17) (table 2). In the group given 8.8 mg/kg of cisplatin without STS and the group given 49.3 mg/kg of cisplatin with STS, there was a slight increase in BUN levels, but these levels returned to normal 13 days after the treatments. On the contrary, when saline was administered instead of STS with 49.3 mg/kg of cisplatin, the mean BUN level was increased to 34.7 mg/dl the next day and eight of 11 mice died of toxicity 4 days after the treatment. The mean BUN level in the remaining three mice increased about fivefold, compared to the untreated controls. Thus, nephrotoxicity of mice treated with a high dose of cisplatin was suppressed with STS, to a minimum level. Table 3 shows the changes in leukocyte counts after cisplatin treatments, with or without STS. In both of the groups given 49.3 mg/kg of cisplatin plus STS and the group given 8.8 mg/kg of cisplatin without STS, there was a slight drop in the leukocyte counts 4 days after the treatments, but the degree was significantly smaller than in eight survivors among 35 mice treated with 49.3 mg/kg of cisplatin without STS. Thus, it is clear that STS protected mice against both nephrotoxic and hematologic toxic effects. The best antitumor effects were observed with cisplatin doses of 8.8 and 49.3 mg/kg for single and combination treatments with 3500 mg/kg of STS, respectively, where only slight side effects were observed. Therefore, these doses seemed optimal in our present mouse model. Thus, the antitumor effect at the optimal dose in TRC is superior to that in the case of single cisplatin treatment. If the optimal dose of 8.8 mg/kg of cisplatin in a single treatment is combined with STS, the antitumor effect may be inferior to 8.8 mg/kg of cisplatin alone, as deduced from the result that the percent increase in life span for 8.8 mg/kg of cisplatin alone was greater than that for 12.3 mg/kg of cisplatin with STS (table 1). We gave 5.6-fold of cisplatin in combination with STS and compared the effect with single cisplatin treatment at equitoxic levels. Consequently, a greater antitumor effect was seen with the former.

DISCUSSION

High doses of STS mixed with cisplatin completely protect bacteria against cisplatin cytotoxicity (11), and the concomitant ip administration of exceedingly high doses of STS with a lethal dose of cisplatin produced no toxicity in mice (10). These observations suggest that STS may combine directly with cisplatin to form a complex no longer capable of reacting with nucleophilic sites of other molecules. Presumably, the complex is $Pt[(S_2O_3)_4]^{6-}$ according to platinum coordination chemistry (18).

Therefore, with our two-route system, it may be speculated that the intraperitoneally disseminated cancer cells come under direct attack by high doses of cisplatin administered ip, while the drug entering the systemic circulation from the peritoneal cavity is antagonized by STS which had been systemically administered. Thus, to achieve complete protection from the side effects of cisplatin, a sufficient amount of STS should be in the systemic circulation when cisplatin enters the blood stream. The results of our present pharmacokinetic study demonstrate that the plasma concentration of platinum after ip cisplatin administration increases rapidly and reaches a peak level at 10 mins after the administration. On the other hand, the peak plasma concentration of STS given sc was much smaller than that of STS given iv, presumably because STS elimination from the blood is more rapid than the absorption into the blood from sc. The

TABLE 2.—Changes in BUN levels after treatments with cisplatin + saline or cisplatin + STS

Treatment	Days after treatment	No. of mice	Mean BUN level ± SE	*P* value*
Untreated control		10	20.8 ± 0.9	
Cisplatin (8.8 mg/kg) ip	1	11	18.3 ± 0.7	< 0.05
+ saline iv	4	10	23.3 ± 2.6	< 0.05 (*a*)
	7	11	22.6 ± 1.2	NS
	10	6	18.6 ± 0.6	NS
	13	7	23.4 ± 1.0	NS
Cisplatin (49.3 mg/kg) ip	1	10	16.7 ± 1.0	< 0.01
+ STS (3500 mg/kg) iv	4	9	22.6 ± 2.0	< 0.05 (*b*)
	7	9	23.6 ± 1.1	NS
	0	6	26.3 ± 2.7	< 0.01
	13	7	23.2 ± 1.1	NS
Cisplatin (49.3 mg/kg) ip	1	11	34.7 ± 2.8	< 0.001
+ saline iv	4	3†	106.2 ± 3.7	< 0.001 (*c*)

* Probability was evaluated by Student's t test: (*a*) and (*b*) vs (*c*) is $P < 0.001$. NS = not significant.
†8 of 11 mice died of cisplatin-induced toxicity 4 days after treatment.

TABLE 3.—Changes in leukocyte count after treatments with cisplatin + saline or cisplatin + STS

Treatment	Days after treatment	No. of mice	Mean leukocyte count + SEM	*P* value*
Untreated control		10	7743 ± 672	
Cisplatin (8.8 mg/kg) ip	1	11	5529 ± 549	NS
+ saline iv	4	11	5388 ± 498	< 0.05 (*a*)
	7	11	6501 ± 500	NS
	10	6	7918 ± 410	NS
	13	7	7151 ± 569	NS
Cisplatin (49.3 mg/kg) ip	1	11	6928 ± 632	NS
+ STS (3500 mg/kg) iv	4	10	5328 ± 574	< 0.05 (*b*)
	7	10	6760 ± 617	NS
	10	6	8190 ± 1140	NS
	13	7	8142 ± 195	NS
Cisplatin (49.3 mg/kg) ip	1	10	4021 ± 468	< 0.001
+ saline iv	4	8†	2612 ± 280	< 0.001 (*c*)

* Probability was evaluated by Student's t test: (*a*) and (*b*) vs (*c*) is $P < 0.001$. NS = not significant.
†27 of 35 mice died of cisplatin-induced toxicity 4 days after treatment.

pharmacokinetic data seem to explain the effective protection of iv STS rather than sc STS administration against cisplatin lethal toxicity in mice (fig 4). The routes of STS administration which involve absorption processes other than sc may be less efficient than STS iv administration in protecting against the cisplatin toxicity. When the iv doses of STS were increased from 1750 to 3500 mg/kg, the LD50 value of cisplatin ip in the group of TRC using cisplatin plus STS increased up to 5.6-fold compared with that in the group of cisplatin treatment without STS. Despite the superiority of the iv route for STS administration, it has to be determined whether additional STS administration is necessary, since the half-life is short in the case of the iv route. When an additional 1750 mg/kg of STS was given iv 20 mins after 3500 mg/kg of STS iv, the ratio of LD50 values of cisplatin ip for the group of TRC (120 mg/kg) to the group of cisplatin without STS (20 mg/kg) showed only a slight increase up to 6.0:1. These observations and results presented in figure 3 suggest that protection from the rapid increase of reactive platinum in the bloodstream during 10 mins after cisplatin ip (fig 1) is crucial to prevent cisplatin-induced toxicity.

TRC in which 3500 mg/kg of STS was given iv 1 min after cisplatin ip did provide significantly better antitumor effects, as evaluated by survival time, than did cisplatin treatment without STS at an equitoxic level, but there was little difference in loss of body weight after the treatments. Peritoneal adhesion due to high concentrations of cisplatin was never observed in autopsied animals. Nephrotoxicity and hematologic toxicity were examined after treatments (using mice without tumor) by measuring BUN levels and leukocyte counts, respectively. These two types of toxicity were suppressed to minimum levels in TRC, even at a high dose of 49.3 mg/kg of cisplatin. In contrast, a high dose of 49.3 mg/kg of cisplatin ip without STS produced severe nephrotoxicity as well as hematologic toxicity. Since STS protects against both nephrotoxic and hematologic toxic effects, STS seems to bind directly to cisplatin in the bloodstream. There is the possibility that other side effects of cisplatin, such as gastrointestinal side effects and ototoxicity (19), can be avoided with STS. This TRC may be applicable not only for intraperitoneal tumor systems (20) but also for other regionally confined tumor systems in man.

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Role of Computerized Cranial Tomography in the Staging of Small Cell Carcinoma of the Lung[1]

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Fifty-five patients with small cell lung cancer underwent computerized cranial tomography (CCT) and a complete neurologic examination as part of their staging work-up. Fifteen patients (27%) had evidence of brain metastases detected by CCT at the time of diagnosis. Eleven of these 15 patients had new focal abnormalities upon neurologic examination. Of the 44 patients who had no new focal abnormalities upon neurologic examination, four (9%) had CCT findings consistent with brain metastases, and, in three of these patients, the central nervous system was the only site of metastatic disease. Thus, through the use of CCT as a routine procedure in the staging of small cell lung cancer, three patients whose tumors would otherwise have been classified as limited-stage were found to have extensive-stage disease. [Cancer Treat Rep 68:1375–1377, 1984]

Central nervous system metastases occur frequently in patients with small cell lung cancer (1). Prior to the advent of computerized cranial tomography (CCT), the neurologic examination and radionuclide brain scan were used as screening procedures. More recently, contrast-enhanced CCT for the detection of intracranial neoplasm has been shown to be superior in sensitivity and specificity to the radionuclide brain scan, and at least comparable to angiography (2-4). The value of CCT as a screening procedure in asymptomatic cancer patients has not been adequately studied in the past (5). To assess the need for routine CCT in the staging work-up of asymptomatic patients with small cell lung cancer, we report the correlation of CCT findings with the neurologic examination in 55 patients with small cell lung cancer.

METHODS

Sixty-nine patients were diagnosed with histologically confirmed small cell lung cancer at the Boston Veterans Administration Medical Center between January 1980 and July 1983. We retrospectively reviewed the charts of these patients, and 55 were found to have undergone a complete neurologic examination and CCT at the time of diagnosis as routine staging procedures. The neurologic examination included the neurologic review of systems, mental status, cranial nerves, motor function, sensation, cerebellar function, gait, reflexes, and Babinski's sign. The presence of new neurologic signs and symptoms constituted an abnormal neurologic exam. Findings which were known to have been present in the past due to non-neoplastic pathology were disregarded. Most CCT scanning was performed on a Technicare Delta Scan 150 with 9-mm section thickness and 2-minute scan time. Iv injection of a 30% solution of meglumine diatrizoate (Reno-M-Dip) was used in 54 patients for contrast enhancement. One patient with a history of allergy to contrast material was scanned without enhancement. Each scan was interpreted by the neuroradiology staff and designated as positive or negative for metastatic disease. CCT criteria for metastasis were those outlined by Jacobs et al (6) and were designed to differentiate between CCT abnormalities due to metastasis and those due to non-neoplastic pathology. To detect other areas of distant metastasis, radionuclide liver-spleen scan, bone scan, and

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bone marrow aspiration or biopsy were routinely performed.

RESULTS

Fifty-five patients had both CCT and a neurologic examination. Sixteen patients had limited-stage disease and 39 had extensive-stage disease. Fifteen patients (27%) had CCT results which were interpreted as positive for tumor metastases. Eleven of these 15 patients had new focal neurologic deficits. In ten of these 11 patients, the abnormalities detected by the neurologic examination were anatomically consistent with the CCT findings. (One patient had a left hemiparesis but a left frontal mass on CCT.) Forty patients had scans which were negative for metastases, and none had new focal abnormalities upon neurologic examination. All patients with new neurologic abnormalities had CCT evidence of metastases. In the group of 15 patients who had intracranial metastases by CCT, six had no other evidence of metastatic disease. Three of these patients had normal neurologic examinations and were considered to have limited disease prior to CCT.

DISCUSSION

The early detection of intracranial metastases has important implications in the management of patients with small cell lung cancer. A review of the literature reveals that CCT is a more sensitive tool for the detection of intracranial metastases at the time of diagnosis of small cell lung cancer than radionuclide brain scan (2-4). Our study further demonstrates that CCT is also more accurate than neurologic examination.

Johnson et al (7) found that two of 60 (3%) neurologically asymptomatic patients had a positive CCT, and in only one patient did the results of CCT change the clinical staging. They state, therefore, that CCT should not be a part of the routine staging procedure in small cell lung cancer. Based on our data, we have reached the opposite conclusion. In our study, four of 44 (9%) neurologically asymptomatic patients had positive findings on CCT, and in three of these patients, routine use of CCT allowed for more accurate clinical staging by demonstrating extrathoracic metastases. In many centers, this information would alter treatment toward that designed to benefit the patient with extensive disease (8).

One could argue that computerized tomography (CT) of the abdomen should also become a routine staging procedure in small cell lung cancer, since previously undetected hepatic or adrenal metastases may similarly alter treatment in some patients. However, the sensitivity of abdominal CT relative to the combined use of liver enzyme assays and liver-spleen scan for the detection of hepatic metastases is unknown. The significance of an adrenal mass detected by CT in a patient with no evidence of endocrine dysfunction is also unclear (9).

All 55 patients in our study were initially staged with CCT, and brain metastases were found in 27% of the patients. Johnson et al (7) also used contrast-enhanced CCT in the staging of small cell lung cancer, and 14% of the patients had brain metastases at the time of diagnosis. Other studies have demonstrated a 9%-14% incidence (10-12). This difference may be related to the frequently advanced disease at presentation in our patient population.

A number of investigators have explored the value of prophylactic whole-brain irradiation in patients achieving complete regression of systemic metastases. These studies have demonstrated a decrease in the incidence of relapse in the central nervous system, although at the expense of considerable morbidity in some patients (13,14). To avoid this problem, Muggia (15) has suggested the possibility that prophylactic brain irradiation may be unnecessary if CCT is used routinely in the staging and restaging of these patients. Baglan and Marks (16) have found that irradiation of patients with brain metastases when they become symptomatic is as effective as prophylactic therapy in achieving palliation of a neurologic deficit, suggesting that there is no role for detecting asymptomatic brain metastases. However, the findings in their study are based on historical comparison, and have yet to be confirmed by other investigators.

In conclusion, at the present time, patients with extensive-stage disease are treated differently from those with limited-stage disease in many centers. Through the use of CCT, three patients in our series whose tumors would otherwise have been classified as limited-stage were found to have extensive-stage disease. Therefore, based on the results of our analysis, we recommend the use of CCT in the routine staging of patients with small cell lung cancer.

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Hepatic Toxicity of Low Doses of Mithramycin in Hypercalcemia[1]

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The medical records of patients receiving mithramycin for the treatment of hypercalcemia at The Johns Hopkins Oncology Center were reviewed. Of 67 evaluable patients, 11 (16%) developed hepatic toxicity when graded using standard criteria. The pattern of laboratory abnormalities noted suggested mild hepatocellular damage that was reversible within 2 weeks. Review of these patients did not identify factors which predispose to hepatic toxicity, although failure to do so may reflect the limited number of toxic patients evaluated. Mild, reversible hepatic dysfunction appears to be a more common sequela of mithramycin administration than has previously been recognized. [Cancer Treat Rep 68:1379–1381, 1984]

Mithramycin is an antineoplastic antibiotic currently used primarily in the treatment of hypercalcemia associated with malignancy (1). Early clinical reports of mithramycin demonstrated that hepatic toxicity, manifested as elevations in enzymes and bilirubin in serum, occurred in virtually all patients treated with the drug in doses of 25–50 μg/kg daily for 5 days (2). This hepatic toxicity, together with the frequent occurrence of renal toxicity, severe thrombocytopenia, and hemorrhagic complications, limited the use of mithramycin as an antineoplastic agent (3). The degree and incidence of liver enzyme abnormalities and hepatotoxicity in patients treated with lower doses of mithramycin for hypercalcemia have not been adequately reported but are believed to be minimal (4).

METHODS

The medication profiles of patients admitted to The Johns Hopkins Oncology Center from 1977 through 1982 were examined. The medical records of patients who had received mithramycin were then reviewed further. A patient was considered to be evaluable for hepatic toxicity if liver function tests (SGOT, SGPT, alkaline phosphatase, and bilirubin) were available within 2 days prior to drug administration and within 7 days after drug administration. Toxicity was graded using the criteria of the Eastern Cooperative Oncology Group (ECOG): *grade 1*—elevations of 1.5 to two times the normal values for these parameters in the clinical laboratory; *grade 2*—elevations from two to five times the normal; and *grade 3*—elevations greater than five times the normal. In patients with abnormal liver function tests prior to receiving mithramycin, a further increase > 1.5 times their own baseline values was required for drug toxicity to be implicated. In addition, we further evaluated patients with normal liver function tests prior to mithramycin therapy who had elevations of greater than two times their own baseline values but who did not reach ECOG grade 1 toxicity (1.5 to two times the upper limit of normal).

RESULTS

During the period studied, there were 67 evaluable patients and 111 evaluable courses of mithramycin given for hypercalcemia. This represents 61% of the patients treated with mithramycin and 34% of the total doses administered during that time. Patients received a single dose of 10–25 μg/kg in each evaluable course. Most patients received total doses between 1000 and 1500 μg.

As shown in table 1, 11 patients (16%) had grade 1, 2, or 3 toxicity. Fifteen additional patients (22%) had greater than twofold increases in their own baseline liver function tests but did not reach ECOG grade 1 toxicity. The most sensitive indicator of mithramycin-induced liver damage appeared to be SGOT, which was elevated in all patients. Of the 11 patients who had grade 1, 2, or 3 hepatic toxicity based on SGOT elevation, only six would have been considered toxic based solely on their SGPT values, although all patients had some corresponding in-

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TABLE 1.—Incidence of hepatotoxicity in patients treated with mithramycin

	No. of patients		
	Myeloma	Nonmyeloma	Total
Total evaluable	31	36	67
Total with hepatotoxicity*			
Grade 1	2	3	5
Grade 2	0	1	1
Grade 3	3	2	5
	5 (16%)	6 (16%)	11 (16%)
Total with enzyme elevations 2 times their baseline but not reaching ECOG grade 1 level	12 (38%)	3 (8%)	15 (22%)
Total	17 (54%)	9 (24%)	26 (38%)

* ECOG criteria.

crease in SGPT. The transaminases (SGOT, SGPT) began to rise within 2 days of drug administration and had returned to baseline within 2 weeks, with one exception. Only two patients would have been considered toxic based on serum alkaline phosphatase elevations, and only one patient would have been considered toxic based on elevated bilirubin. There were no increases in the alkaline phosphatase values in six of these toxic patients, and no increase was seen in the bilirubin of seven patients. The remainder of the patients had minimal increases in these laboratory tests which did not reach either ECOG toxicity or 1.5 times their own baseline.

In eight of the 11 toxic patients the toxicity occurred during initial exposure to the drug. Six of these eight patients required additional therapy, with an interval between treatments ranging from 2 days to 2 weeks. Toxicity reoccurred in four of these six patients but did not appear to increase in severity. However, based on this small number of patients, no conclusions are possible concerning whether this toxicity is cumulative or whether it would be more severe with a short interval between drug doses. Five of 15 (33%) patients with breast cancer developed hepatotoxicity as compared to five of 31 (16%) patients with multiple myeloma and one of 21 (4%) with miscellaneous solid tumors. The median age of toxic patients was no different from that of patients who did not encounter adverse drug effects (67 [range, 35–79] vs 61 [range, 17–83]; $P = 0.2$). No differences in the concomitant drugs being administered to patient groups could be discerned. The presence of hepatic metastases or mild pretreatment liver function test abnormalities did not appear to predispose patients to toxicity.

The renal toxicity associated with mithramycin was not seen in this group of patients. Of the 26 patients in this study with elevated liver function tests, only three had simultaneous rises in serum creatinine ranging from 1.5 to two times baseline and resolving in 2–3 days. These patients were not evaluable for thrombocytopenia because of the frequency with which concurrent cytotoxic therapy was applied.

DISCUSSION

Although the consistent and occasionally severe hepatic toxicity noted in the early studies with mithramycin has not been seen in patients treated with lower doses for hypercalcemia, a review of patients at our center indicates that mild hepatic dysfunction commonly follows mithramycin administration. Using a standard cooperative group grading scale for toxicity, 16% of the patients developed a pattern of hepatic toxicity consistent with mild hepatocellular damage. Elevations of alkaline phosphatase and bilirubin were much less common. An additional 22% of the patients doubled their pretreatment transaminase values.

The molecular mechanism by which mithramycin exerts its toxic effects on target cells is not known, although it is clear that the drug binds to DNA and at appropriate concentrations can inhibit RNA, DNA, and protein synthesis (1). It is, presumably, one of these effects that results in the central lobular hepatic necrosis that has been seen in man (2). The incidence and degree of the toxicity seem clearly to be dose-related, based on the lower incidence noted in this report.

A review of our experience with this drug does not provide a clear indication of which patients are at risk for mithramycin toxicity on initial exposure, nor does it define those at risk for further injury on subsequent exposures. These data do suggest that, as the drug is currently used, hepatic toxicity is more common than has previously been recognized but is mild and reversible.

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Evaluation of a Sequential 5-FU and Hydroxyurea Combination in Advanced Bowel Cancer[1,2]

A. K. Kao, F. M. Muggia,* N. Dubin, W. A. Lerner, R. Stark, J. C. Wernz, J. L. Speyer, and R. H. Blum[3]

Twenty-nine patients (two with small bowel cancer and 27 with colorectal cancer) were treated with a sequential 5-FU-hydroxyurea combination following the suggestion of schedule-dependent synergism in experimental systems. No enhanced toxicity was observed, but the response rate was only 4%. Seven additional patients manifested ≥ 50% declines in CEA, but caution must be used in interpreting such changes as antitumor activity. [Cancer Treat Rep 68:1383–1385, 1984]

The mechanisms for 5-FU cytotoxicity are unclear. Two major possibilities exist: (*a*) incorporation into RNA by conversion of 5-FU to 5-fluorouridine triphosphate (FUTP); and (*b*) inhibition of DNA synthesis by activation of 5-FU to FdUMP and competition with dUMP as substrate for thymidylate synthetase. The correlation of tumor sensitivity to persistence of FdUMP levels in some cells (1) lends support to the latter hypothesis.

Theoretically, one mechanism limiting the activity of 5-FU is the accumulation of dUMP, which competes with FdUMP for the target enzyme. Expansion of the dUMP pool may occur via the decreased feedback inhibition of ribonucleotide reductase by decreased levels of nucleoside triphosphates (2). Inhibitors of ribonucleotide reductase, such as hydroxyurea (HU) or guanazole, have enhanced the antitumor activity of 5-FU in several experimental systems (3,4). More recently, Kobayashi and Hoshino (5) demonstrated potentiation of cytotoxicity of 9L rat brain tumor cells by HU when given *after* subtoxic doses of 5-FU. Such potentiation occurred with other ribonucleotide reductase inhibitors and was blocked by concomitant thymidine. These experiments suggest that drugs that lower dUMP levels may potentiate 5-FU effects and that this enhancement is schedule-dependent. Although several trials using different doses and schedules of 5-FU and HU (table 1) have been reported, none has used the sequence which was suggested as optimal in preclinical studies. Therefore, in spite of variable results from previous studies, we initiated an evaluation of this combination in advanced colorectal cancer utilizing a sequential schedule. Our objective was to search for greater antitumor activity than that of 5-FU alone, without greater toxicity.

PATIENTS AND METHODS

Twenty-nine patients with a histologically confirmed diagnosis of unresectable, residual, recurrent, or metastatic bowel cancer previously untreated with 5-FU were entered in the study at the New York University Medical Center between August 1981 and May 1983.

The treatment schedule involved 5-FU at a dose of 450 mg/m^2 iv for 4 days followed by HU at a dose of 800 mg/m^2 every 8 hrs × 6 doses (ie, administered orally on Days 4 and 5, initiated 4 hrs following the last 5-FU dose). The cycle was repeated every 28 days. Doses of 5-FU were decreased by 20% for either grade 3 or grade 4 hematologic and gastrointestinal toxic effects (Eastern Cooperative Oncology Group toxicity criteria).

Twenty-seven patients had colorectal cancer and two had small bowel cancer. There were 20 men and nine women with a median age of 64 yrs (range, 31–82). Only five patients had received prior radiotherapy (one patient) or chemotherapy (three patients had a combination of mitomycin and methotrexate and one patient had 5 days of adjuvant 5-FU). Sites of metastasis included: liver (21 patients), lung (six), brain (three), spine (one), peritoneum (four), and lymph node (three). The only measurable manifestation of disease in five patients was a positive CEA titer (three patients had known liver metastases, and two had known peritoneal disease at surgery).

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TABLE 1.—Clinical trials combining 5-FU and HU in the treatment of metastatic colorectal cancer

Investigator (ref No.)	Schedule	No. of patients treated	Results
Lerner (6)	HU orally, 80 mg/kg, Day 1; 5-FU iv or orally, 0.5 g, Day 4 weekly	20	Median survival of 620 days
Lokich et al (7)	HU orally, 80 mg/kg, Day 1; 5-FU iv, 500 mg/m^2, Day 2 weekly	19	No response; increased neurotoxicity
Krein et al (8)	5-FU iv, 12 mg/kg, Day 1; HU orally, 50 mg/kg, Day 4 weekly	21	Median progression-free survival of 12 mos
Lavin et al (9)	5-FU iv, 600 mg/m^2, Day 1; HU orally, 800 mg/m^2, q 8 hrs, Day 4 weekly	94	21% response rate; 35-wk median survival
Kao et al (present study)	5-FU iv, 450 mg/m^2, Days 1-4; HU orally, 800 mg/m^2, q 8 hrs, Day 4, 5 cycles repeated q 28 days	29	4% response rate;* 24% response rate;† 12-mo median survival

*Partial response by tumor measurements only.
†Including responses measured by CEA only (*see* text).

RESULTS

Therapeutic Response

The median total number of cycles per patient was five (range, one to 13). Among 24 patients with objective measurable disease, there was one partial response lasting 3 mos. This response occurred in a patient with colon cancer and was documented by a reduction in the size of a palpable liver, sequential ultrasound recordings, and a > 50% decrease in previously elevated CEA titer. Four patients have had stable disease for 4, 5, 6, and 9 mos. Eight patients who developed progressive disease are still alive; 11 have died of disease progression. Median survival for the entire group was 12 mos from onset of therapy.

Seven patients demonstrated a ⩾ 50% reduction in plasma CEA level. The fall in CEA was noted in two patients with stable disease and in the one partial responder. In addition, four of the five patients in whom the CEA level was the only evaluable parameter showed a decline in CEA, which persisted for 3, 5, 8, and 20+ mos.

Toxicity

Toxicity with this treatment program was minimal. Gastrointestinal intolerance was mild and responded to self-administered symptomatic therapy; 45% of the patients developed leukopenia (wbc count < 4000/mm^3 but none < 2000/mm^3), one patient developed thrombocytopenia (platelet count < 100,000/mm^3), and one developed anemia (hemoglobin decrease of > 2 g/100 ml). There were no blood transfusions required, no complications of myelosuppression (sepsis or bleeding), and no treatment-associated deaths. Vomiting occurred in 17% of the patients, diarrhea in 27%, stomatitis in 17%, and alopecia in 10%. Neurologic toxicity was not recorded.

DISCUSSION

Although the regimen does not appear to enhance 5-FU toxicity, the response rate is disappointing and probably does not differ from that observed when 5-FU is used as a single agent or in analogous combinations for the treatment of bowel cancer. The response of patients whose evaluation was based on CEA levels only is noteworthy. Four of five such patients had documentation of a ⩾ 50% reduction of CEA levels. Three patients with measurable disease also manifested this reduction, including two who continue stable and the one who had an objective response. If this criterion were to be used to determine response, a treatment might be considered to have greater activity than if only direct tumor measurements are used. In fact, defining responses by CEA decline and absence of disease progression would yield a response rate of 24% (seven of 29 patients; 95% confidence interval, 10%-43%). However, proper interpretation of this result requires further study of CEA as an independent variable in the assessment of response to chemotherapy in bowel cancer.

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Ineffectiveness of Scalp Hypothermia in the Prevention of Alopecia in Patients Treated With Doxorubicin and Cisplatin Combinations[1]

John B. Wheelock,* Margaret B. Myers, Hans-B. Krebs, and Dean R. Goplerud[2]

Eleven patients were studied to determine the effectiveness of scalp hypothermia in the prevention of chemotherapy-induced hair loss. Scalp hypothermia was induced by the Kold Kap. The cytotoxic regimen used consisted of either cisplatin, doxorubicin, and cyclophosphamide or cisplatin, doxorubicin, and methotrexate given at monthly intervals. All 11 patients required a wig by the end of the fifth course of chemotherapy because of severe hair loss. Ten of the 11 patients (91%) had severe hair loss by the end of the second course of chemotherapy. These results suggest that scalp hypothermia as used in this study is ineffective in preventing chemotherapy-induced alopecia in patients treated with cisplatin and doxorubicin drug combinations in doses commonly used for treatment of gynecologic malignancies. [Cancer Treat Rep 68:1387–1388, 1984]

Alopecia is a temporary but most disturbing side effect of many cytotoxic drug regimens. Although physically not impairing, complete hair loss may be socially very disturbing for female patients, despite the availability of artificial hair pieces. Various devices ranging from ice packs to disposable hats have been tried to provide temporary vasoconstriction of local blood vessels, thereby reducing the exposure of the hair follicle to the chemotherapeutic agents known to cause alopecia. A recent report has demonstrated good or better protection (63%) in preventing alopecia in patients exposed to doxorubicin or combinations of drugs that include doxorubicin (1). In patients with gynecologic malignancies, the rapidity and completeness of hair loss while undergoing combination chemotherapy that included doxorubicin and cisplatin prompted our trial of scalp hypothermia in hopes of preventing or delaying alopecia.

METHODS

All patients in the Gynecologic Oncology Service of the Medical College of Virginia receiving combination chemotherapy consisting of either cisplatin, doxorubicin (Adria Laboratories, Inc, Dublin, OH), and cyclophosphamide or cisplatin, doxorubicin, and methotrexate were eligible for entry in the study unless they had (*a*) significant liver disease, (*b*) scalp metastasis, (*c*) prior baldness, (*d*) irradiation to the scalp, or (*e*) cryoglobulinemia. Of the 11 patients studied, five had ovarian cancer and were treated with cisplatin, doxorubicin, and cyclophosphamide. The same regimen plus medroxyprogesterone acetate (40 mg orally twice a day) was used in two patients with recurrent endometrial carcinoma. Four patients with recurrent cervical carcinoma were treated with cisplatin, doxorubicin, and methotrexate. The chemotherapy was given every 4 weeks at doses of 50 mg/m^2 for cisplatin and doxorubicin, 500 mg/m^2 for cyclophosphamide, and 20 mg/m^2 for methotrexate. Cisplatin was given by injection over 6 hours; all other drugs were given by iv push while scalp hypothermia was applied.

The average dose of cisplatin was 72 mg; doxorubicin, 72 mg; cyclophosphamide, 725 mg; and methotrexate, 25 mg.

No patient had received prior chemotherapy. Informed consent was obtained from all patients. The Kold Kap (American Kaye Laboratory, San Diego, CA) was used for induction of scalp hypothermia in accordance with the manufacturer's directions, which included moistening the hair, covering the ears with enclosed ear protectors, and applying the cap 15 minutes prior to administration of the chemotherapy. The cap was held in place by an elastic bandage wrapped around the head and was left in place for 45 minutes, after which time it was removed.

The amount of hair loss was evaluated at the next course of chemotherapy by one of the investigators (M. Myers) and the patient. Hair loss was defined as minimal

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or moderate if < 25% or 50%, respectively, of the hair was lost and if the patient did not find it necessary to use a wig or hat. Severe hair loss was defined as a > 50% loss of hair and/or the patient found it necessary to wear a wig or hat.

RESULTS

All patients had severe hair loss before completion of the chemotherapy and found it necessary to wear a wig or hat. Eight patients required wearing a wig or hat after the first course of chemotherapy, two patients after two courses, and one patient after five courses. Thus, in all patients but one, severe hair loss necessitating the wearing of a wig or hat occurred within 2 months of initiation of chemotherapy.

No severe side effects to the Kold Kap were seen. Most patients complained of feeling cold. This symptom was readily relieved with the use of blankets after discontinuation of the scalp hypothermia. Two patients complained of a headache despite adequate support of the head and neck with pillows. One patient complained of claustrophobia. Because of the uniformly poor results in preventing hair loss by the Kold Kap, the study was terminated at 11 patients.

DISCUSSION

Alopecia is a common but most disturbing side effect of many chemotherapy regimens. Although usually temporary and physically not impairing, it may be the cause of considerable anxiety for the patient and a reason to refuse chemotherapy and forfeit possible cure of the disease. Most patients will accept complete hair loss and prepare themselves by purchasing a wig, hat, turban, or scarf to cover the head until regrowth of the hair occurs.

Almost invariably, chemotherapeutic agents such as doxorubicin and vincristine will cause alopecia (2). Other agents such as methotrexate and cyclophosphamide may cause alopecia although complete hair loss is uncommon. Cisplatin is not known for causing hair loss (2).

Most techniques to minimize, delay, or prevent hair loss during chemotherapy are aimed towards decreasing blood flow to the scalp, thus reducing the concentration of the chemotherapeutic agent to the hair follicle. Scalp tourniquets have not proven to be effective (3). Scalp hypothermia is another concept of decreasing blood flow to the scalp by vasoconstriction. Ice packs with crushed ice applied to the scalp during the administration of the chemotherapy have been tried with good success (4). A refinement of the ice pack is the disposable pack that uses an endothermic reaction of amonium nitrate and water to produce the cooling effect. Such a device is the Kold Kap, which produces a chilling effect < 32°C for 90 minutes that is constant, uniform, and without the inconvenience of the crushed ice (1). Reduced biochemical activity at a temperature < 32°C may be more important in preserving hair than the hypothermic-induced vasoconstriction (5).

Dean et al (1) compared the Kold Kap with their previously published results using crushed ice. The success rate in that series was 63%. Satterwhite and Zimm (6) reported acceptable hair preservation in 75% of patients studied in their series. The results from these studies contrast sharply with our experience using the Kold Kap in patients undergoing chemotherapy with doxorubicin and cisplatin combinations. Although the manufacturer's instructions were followed meticulously, none of the patients derived significant benefits from the Kold Kap since all patients had severe hair loss after five courses of chemotherapy and 91% (ten of 11 patients) after two courses of chemotherapy. It is possible that the higher dose of doxorubicin [50 vs 30 mg/m^2 according to the study by Dean et al (4)] created decisively higher drug levels at the hair follicle to render the Kold Kap ineffective. The combination of doxorubicin with cisplatin and cyclophosphamide or methotrexate may also be significantly more toxic for hair follicles than doxorubicin alone or in combination with drugs other than the drugs used in this study. Finally, the prolonged infusion of cisplatin well beyond applications of the Kold Kap may have been responsible for the poor results obtained with scalp hypothermia in this study, although it is generally believed that cisplatin will not cause alopecia.

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Doxorubicin and Cisplatin in the Treatment of Advanced Mixed Mesodermal Uterine Sarcoma[1]

Vicki Seltzer,[2,*] Barry Kaplan,[3] Steve Vogl,[3] and Mark Spitzer[2]

Mixed mesodermal sarcoma of the uterus is a very aggressive malignancy. Approximately 50% of patients who initially present with stage I tumors will ultimately develop recurrent disease and succumb to the lesion. For patients presenting with disease more advanced than stage I, survival for > 1 year is uncommon.

Attempts to cure advanced and recurrent disease with chemotherapy and/or radiation have met with limited success. Hannigan et al (1) reported only a 10.3% response rate for patients treated with doxorubicin for advanced uterine sarcoma, and there were no complete responses. Using vincristine, dactinomycin, and cyclophosphamide, their response rate was 28.3% (13.3% complete, 15% partial) (1). A Gynecologic Oncology Group trial evaluated doxorubicin, alone or in combination with dacarbazine, and found that the response rate for advanced or recurrent mixed müllerian sarcomas was < 25% with either regimen, and the duration of response was brief (2).

Since uterine sarcomas have been demonstrated to have some response to doxorubicin, sarcomas in other areas of the body have been noted to respond to cisplatin (3), and the combination of doxorubicin plus cisplatin has been found to be effective in the treatment of other varieties of malignancies (4), we treated six sequential patients who presented with advanced or recurrent uterine mixed mesodermal sarcomas with the combination of doxorubicin (50 mg/m²) and cisplatin (50 mg/m²) iv every 3 weeks (table 1).

Of the six patients treated, three had complete responses, and two of these are still in remission. Two patients had partial responses, one of whom is still in remission. Only one of six women did not respond to therapy. Toxicity has been minimal, consisting of mild to moderate bone marrow suppression for all patients. The duration of response for our complete responders has been 9, 9+, and 11+ months, which is longer than has been noted with other chemotherapeutic agents which have been used to treat this lesion (1).

The combination of doxorubicin and cisplatin in the treatment of advanced mixed mesodermal uterine sarcomas appears to have significant activity. Since the capability of producing a response in women with this lesion has been quite limited, this combination of drugs in the treatment of advanced uterine sarcoma seems to merit futher investigation.

TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Stage	Initial therapy*	Distribution of disease	Disease-free interval (mos)	Location of recurrence	No. of courses of chemo-therapy	Response†	Duration of response (mos)	Duration of survival (mos)
1	78	IV	Laparotomy and biopsies	Pelvis, upper abdomen	—	—	6	CR	11+	11+
2	74	III	D and C	Pelvis	—	—	6	CR	9	13
3	59	IV	TAH-BSO	Pelvis, upper abdomen	—	—	9	CR	9+	9+
4	67	I	4000 rads to pelvis, TAH-BSO	Uterus	12	Lung, brain	5	PR	6	9
5	54	IV	Biopsy	Pelvis, lung	—	—	2	PR	2+	2+
6	65	III	TAH-BSO	Uterus, ovaries	1	Pelvis	3	DP	—	4

*D and C = dilatation and curettage; TAH = total abdominal hysterectomy; BSO = bilateral salpingo-oophorectomy.
†CR = complete response; PR = partial response; DP = disease progression.

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4′-Epi-doxorubicin in Combination With Cisplatin in Advanced Ovarian Cancer[1]

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Thirty-two patients with advanced ovarian cancer were treated with a combination of 4′-epi-doxorubicin, a new analog of doxorubicin, and cisplatin at a dose of 60 and 50 mg/m², respectively, every 28 days. Twelve patients had previously received chemotherapy and/or radiotherapy. Of 29 evaluable patients, six (21%) achieved complete remission for a median duration of 19+ months, and 13 (45%) achieved partial remission for a median duration of 8 months. All complete remissions were confirmed by a "second-look" procedure. Main side effects included vomiting in all patients, moderate myelosuppression (67%), and alopecia (65%). No case of congestive heart failure was observed. 4′-Epi-doxorubicin plus cisplatin is an effective and relatively well-tolerated regimen in the treatment of advanced ovarian cancer. [Cancer Treat Rep 68:1391–1393, 1984]

4′-Epi-doxorubicin (4′-epi-DX) is an analog of doxorubicin (DX) that has been shown to have acute toxicity and a cumulative cardiotoxicity lower than that of the parent drug in clinical phase I and phase II studies (1). We have previously studied 4′-epi-DX as a single agent (2) and later in combination with cisplatin (CDDP) in patients with advanced solid tumors (3). This report concerns the updated results of this combination in the treatment of advanced ovarian cancer.

MATERIALS AND METHODS

Thirty-two patients were entered in the study between July 1981 and July 1983. Patient characteristics are shown in table 1. A total of 24 patients had measurable disease, and eight with stage III had nonmeasurable residual disease. Previous chemotherapy included alkylating agents in seven patients and multidrug regimens containing DX (total dose < 200 mg/m²) in two. To enter the trial, patients had to have disease progression, life expectancy > 2 months, and normal renal, liver, marrow, and cardiac functions.

Upon entry in the study, each patient underwent a clinical examination, blood cell count, biochemical screen, abdominal and pelvic ultrasounds, ECG, chest x-rays, and cytology of ascites or pleural fluid if present. Both drugs were administered by iv bolus in 5 minutes, and 4′-epi-DX preceded CDDP by 1 hour. A standard 2-hour hydration program with mannitol was performed. 4′-Epi-DX was administered to six patients at 40 mg/m² and to two patients at 50 mg/m². The rest of the patients received 60 mg/m². These doses were the same in the subsequent courses.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	32
Median age in yrs (range): 52 (26–68)	
Median performance status (range): 60 (40–90)	
Stage	
III	11
IV	11
Recurrence	10
Tumor size	
< 2 cm	9
> 2 cm	23
Histology	
Cystoadenocarcinoma	24
Undifferentiated carcinoma	7
Endometrial carcinoma	1
Prior treatment	
Surgery	
Biopsy only	7
TAH + BSO*	13
TAH + BSO + omentectomy	9
Oophorectomy	3
Radiation therapy	3
Chemotherapy ± radiation therapy	9

*TAH + BSO = Total anterior hysterectomy + bilateral salpingo-oophorectomy.

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quent courses. The dose of CDDP was 50 mg/m^2 for all patients. The next courses of therapy were repeated after a minimum of 28 days. The patients were examined monthly, and the treatment continued until disease progression or a maximum of 12 courses.

Complete remission (CR) was defined as the disappearance of all evidence of disease as judged by careful clinical evaluation (physical examination, ultrasounds, and computerized axial tomography) for at least 1 month. Partial remission (PR) was defined as a > 50% reduction in the product of the diameters of all measurable tumor lesions together with a disappearance of ascites and/or pleural effusion for at least 1 month. Stabilization of disease was defined when a 50% decrease in total tumor size could not be established or a 25% increase in the size of one or more measurable lesions was not demonstrated. Disease progression was defined as a > 25% increase in the size of one or more tumor lesions or the appearance of new lesions. Durations of responses and survival were measured from the beginning of therapy. A "second-look" procedure was considered after at least six courses of therapy in patients who achieved clinical CR of measurable lesions, and in patients with nonmeasurable disease at the onset of therapy who had not progressed during the first six courses.

RESULTS

The median number of courses of therapy administered was six (range, two to 12). The median cumulative dose of 4′-epi-DX and CDDP was 320 mg/m^2 (range, 80–720) and 300 mg/m^2 (range, 100–650), respectively. The cumulative dose of 4′-epi-DX was > 420 mg/m^2 in ten patients.

Tables 2 and 3 show the analysis of objective response. Fifteen patients (62%) with measurable disease achieved an objective remission (CR + PR). The two patients who achieved clinical CR had an intra-abdominal disease. These patients had undergone a second-look operation after six and seven courses of therapy, respectively, and no macroscopic or microscopic evidence of disease was noted at laparotomy. Of eight patients with nonmeasurable disease, one had evidence of progression after five courses of therapy, and three are not evaluable because a second look was still not carried out, since they were without evidence of disease at this time. Four patients underwent a second-look procedure (two had laparotomy and two had laparoscopy) after six to eight courses, and no evidence of disease was found. Consequently, of a total of 29 evaluable patients, the CR rate was 21% (six patients) and the PR rate was 45% (13 patients), making a total remission rate of 66%. Five of the six complete responders had not received any prior antitumor treatment. However, a CR + PR occurred in 55% of the patients pretreated with chemotherapy and/or radiotherapy. One of the two patients previously treated with DX achieved PR. At this time, four of six patients are without evidence of disease 8+ to 14+ months following a negative second look. Of eight patients who received 4′-epi-DX at single doses of 40 or 50 mg/m^2, only three showed PR. Median survival of the whole group of the treated patients is 15 months (range, 2–30+). Statistical analysis (generalized Wilcoxon test) shows that survival depends on the dimension of residual disease: of nine patients with residual tumor < 2 cm, only one is dead, in comparison with 15 of 23 with tumor > 2 cm (median survival, 11 months). Similarly, complete plus partial responders had a survival superior to nonresponders (median survival, 16 vs 9 months, respectively), but we cannot state if response causes this difference or if response identifies patients with pretreatment characteristics which favor longer survival.

All 32 patients are evaluable for toxicity. Leukopenia (wbc count < 4000/mm^3) occurred in 21 patients (67%), but only five (16%) had a wbc count nadir between 1000 and 2000/mm^3 and none had a wbc count < 1000/mm^3. Mild thrombocytopenia (platelet count of 75,000–99,000/mm^3) was seen in only two patients (6%). Anemia (Hgb < 11 g/100 ml) was observed during treatment in 14 patients (45%), but in only two (6%) was the Hgb level < 8 g/100 ml. All patients experienced moderate or severe vomiting lasting an average of 2 hours on the day of therapy. Twenty patients (65%) had alopecia, four (12%) had diarrhea, two (6%) had fever, and two (6%) had local toxicity due to extravasation of 4′-epi-DX. Five patients (16%) had a mild and transient increase of serum creatinine (1.6–2.3 mg/100 ml) that did not prevent the continuation of the treatment. Clinical hearing loss, peripheral neuropathy, and congestive heart failure were not observed. The monitoring of ECG showed only minimal and transient changes (tachycardia in one patient, atrial premature beats in two, and flattening of T wave in four). In seven patients, left ventricular ejection fraction determined by radionuclide angiography at rest did not show

TABLE 2.—Analysis of objective responses in patients with measurable disease

Response	No. of patients (%)	Median duration in mos (range)
CR	2 (8)	19 (19–19+)
PR	13 (54)	8 (3–14)
No change	6 (25)	6 (3–6)
Disease progression	3 (12.5)	
Total	24	

TABLE 3.—Analysis of objective responses in patients with nonmeasurable disease

Response	No. of patients	Duration (mos)
CR	4	11, 18+, 22+, 25+
Disease progression	1	
NE*	3	10+, 10+, 23+
Total	8	

*NE = patients not evaluable for response but without evidence of disease.

significant changes after 300–360 mg/m² of 4′-epi-DX, as compared with basal values.

DISCUSSION

The combination of 4′-epi-DX and CDDP at a dose of 60 and 50 mg/m², respectively, has induced objective remission in 29 patients (66%) with advanced ovarian cancer. Twenty-one percent of the patients achieved CR for a median duration of 19+ months, and 45% achieved PR for a median duration of 8 months. In previously pretreated patients, the remission rate was 55%. As a single agent, CDDP induces a response rate of 26% in previously treated patients (4) and 31% in untreated patients (5). Recently, 4′-epi-DX was reported to induce objective remission in 13 of 41 (32%) of the patients with advanced ovarian cancer, the remission rate being 40% in nonpretreated patients and 18% in pretreated patients (6). The combination of CDDP and DX at doses similar to those used in this study induced objective remission in 42% and 55% of two series of patients, the majority having been pretreated (7,8). In a series of 18 untreated patients, this regimen achieved a response rate of 67.7%, which was significantly better than that achieved with CDDP alone in the same study (5). Our results are very similar to those achieved with the combination of CDDP and DX and indicate that the combination of 4′-epi-DX + CDDP could be more effective than CDDP or 4′-epi-DX alone. To verify the possibly better therapeutic index of this regimen containing the new anthracycline, a comparative trial with the regimen containing DX is suggested.

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Phase II Trial of Vinblastine, Bleomycin, and Cisplatin (VBP) Followed by Dacarbazine and Mitolactol in Metastatic Melanoma[1,2]

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The treatment of metastatic malignant melanoma has been unsatisfactory. Dacarbazine (DTIC) and combinations containing DTIC have been the mainstay of therapy for metastatic melanoma, although most response rates have not exceeded the range of 20%–30% (1).

Nathanson et al (2) reported that a regimen consisting of vinblastine, bleomycin, and cisplatin (VBP) resulted in a 70% response rate among 20 patients with malignant melanoma. An update of these data involving the treatment of 42 patients with VBP revealed a 43% response rate, with complete responses observed at visceral sites (3). Because of the limitations inherent in administering bleomycin and because the VBP regimen omits several moderately active agents, we decided to evaluate a sequential regimen of VBP and DTIC and mitolactol (DBD). A 20% response rate has been reported for DBD in 25 patients with melanoma who received 100 mg/m^2/day until hematologic toxicity occurred, following failure on primary protocols containing DTIC and nitrosoureas (4). We elected to use this 10-day regimen combined with the 1-day DTIC schedule (5).

METHODS AND MATERIALS

The treatment plan consisted of an induction phase containing vinblastine (6 mg/m^2) on Days 1 and 2, bleomycin (15 units/m^2) on Days 1–5 as a 24-hour infusion, and cisplatin (50 mg/m^2) on Day 5 preceded and followed by mannitol infusion. The induction regimen was to be repeated at 28-day intervals for two courses. Because of toxicity only the first six patients received the planned dose of vinblastine. All subsequent patients received 3 mg/m^2 on Days 1 and 2. Following the induction regimen the patients were placed on a maintenance regimen consisting of DTIC (800 mg/m^2 iv) on Day 1 and DBD (125 mg/m^2 orally) on Days 1–10, to be repeated every 28 days until progression. Written informed consent was obtained from all patients. Because of the requirement for continuous infusion of bleomycin, all induction regimen courses required hospitalization. Eligibility requirements consisted of histologic confirmation of metastatic malignant melanoma, measurable disease, expected lifespan > 2 months, and creatinine clearance > 60 ml/minute. The protocol had no age restrictions. Patients who had received prior chemotherapy including regional perfusion, systemic chemotherapy, or prior radiotherapy were accepted.

Standard criteria were used to define response. All patients entered were considered evaluable. Response, duration, and survival were measured from Day 1 of treatment to progression and death, respectively.

RESULTS

Twenty patients were entered and all were evaluable. The median age was 56 years. Four patients were < 30 and three were > 70 years of age. Four patients had received prior systemic chemotherapy and in all four, treatment had included DTIC or DBD. Sites of metastatic disease on entry and response sites are listed in table 1. The overall response rate was 35% and consisted entirely of partial remissions. Median survival was 28 weeks. Twenty percent of the patients had stable disease and 45% had disease progression. Median survival for patients with stable disease was 32 weeks and for patients with disease progression was 8 weeks. Two of the six patients (33.3%) receiving high-dose vinblastine (6 mg/m^2) responded, as well as five of the 14 patients (35.6%) receiving low-dose vinblastine (3 mg/m^2). Sites of response were confined to cutaneous-subcutaneous tissue, nodes, and lungs. No patients with visceral disease other than lung responded. Relapses in two patients involved the central nervous system: one in the brain and one in the spinal cord. No responding patient responded further

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TABLE 1.—Patient characteristics and sites of response

	No. of patients	No. of patients responding at site
Sex		
Male	12	
Female	8	
Median age in yrs (range): 56 (24-73)		
Primary site		
Extremity	8	
Trunk	6	
Head-neck	4	
Ocular	1	
Unknown	1	
Prior therapy		
Immunotherapy	2	
Chemotherapy		
Perfusion	4	
Systemic	4	
Sites of metastatic disease		
Cutaneous-subcutaneous	10	3
Lung	7	3
Nodes	6	3
Liver	4	
Bone	4	
CNS	1	
Other	2	

during the maintenance regimen of DTIC and DBD. No patient who failed to respond to VBP responded to DTIC and DBD.

The major toxic effect was myelosuppression. On the VBP induction arm, the median nadir wbc and platelet counts were 2.6/mm^3 (range, 0.2-6.9) and 105/mm^3 (range, 10-417). On the DTIC and DBD arm, the median nadir wbc and platelet counts were 5.2/mm^3 (range, 3.2-10.6) and 276/mm^3 (range, 28-387). Three patients developed sepsis during induction, all following high-dose (6 mg/m^2) vinblastine. Sepsis was not fatal to any of these patients. One patient developed fatal pulmonary fibrosis during the second course of VBP which was interpreted clinically, radiologically, and histologically as consistent with bleomycin toxicity. No nephrotoxicity was observed.

DISCUSSION

Because the dose of vinblastine was reduced from 6 to 3 mg/m^2, this study is not directly comparable to those studies using the higher dose. This study achieved a response rate of 35% consisting entirely of partial responses in the cutaneous-subcutaneous tissue, nodes, and lung. In contrast to Nathanson's report, we did not see significant responses in nonpulmonary visceral disease. Similar results have been reported by Creagan et al (6) and Bajetta et al (7).

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Treatment of Metastatic Ovarian Carcinoma With Amsacrine: A Cancer and Leukemia Group B Phase II Study[1,2]

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Amsacrine (*m*-AMSA), synthesized by Cain and Atwell (1), was selected in 1977 for human studies due to encouraging activity in animal tumors. In phase I trials the dose-limiting toxic effect was myelosuppression (2,3). Because some antitumor activity against ovarian carcinoma was demonstrated in the one trial (3), the Cancer and Leukemia Group B initiated this study in 1979.

PATIENTS AND METHODS

Patient Selection

Between June 1979 and April 1980, 34 patients were entered in this study, and two were disqualified because of low blood cell counts prior to treatment (table 1). All patients were required to have measurable, metastatic, histologically documented ovarian carcinoma, wbc count $> 4000/\mu l$, platelet count $> 100,000/\mu l$, bilirubin < 3.0 mg/dl, and SGOT less than three times normal.

Drug Administration

m-AMSA was initially administered by iv bolus at a dose of 60 or 120 mg/m^2, depending on the extent of prior chemotherapy and radiotherapy. Treatments were given every 3 weeks, and doses were increased by 20 mg/m^2 with each treatment until toxicity was encountered or until a level of 160 mg/m^2 was achieved.

Criteria for Response

Disease was measured by palpable masses and by ultrasound, computed tomography, and other radiographic studies. Standard response criteria were used (2).

TABLE 1.—Pretreatment patient characteristics

Characteristic	No. of patients (%)
No. of evaluable patients	32
Median age in yrs (range): 58.5 (36-79)	
Performance status	
Normal or minor impairment	9 (28)
Bedridden < 50% of time	15 (47)
Bedridden > 50% but < 100% of time	7 (22)
Bedridden 100% of time	1 (3)
Site of tumor masses	
Abdomen	30 (94)
Lung or pleural effusion	14 (43)
Liver	6 (19)
Skin	3 (9)
Nodes	3 (9)
Bone	2 (6)
Other (1 patient each)	6 (19)
Prior therapy	
Surgery	28 (88)
Radiotherapy	17 (53)
Chemotherapy	32 (100)
Single-drug	18 (56)
Combination	31 (97)

RESULTS

Response

Among the 32 patients evaluable for response, there was one (3%) partial response (table 2). This patient had widespread abdominal disease documented at oophorectomy and had failed two prior regimens of chemotherapy. She had an almost complete remission of palpable abdominal masses for 12 months while receiving doses of

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TABLE 2.—Results

	No. of patients (%)
Toxicity	
Wbc count nadir (cells/μl)	
2000–3000	8 (25)
1000–2000	2 (6)
< 1000	5 (16)
Platelet count nadir (cells/μl)	
20,000–50,000	5 (16)
< 20,000	0
Nausea and vomiting	
None	22 (69)
Mild	5 (16)
Moderate	5 (16)
Drug administered	
Initial dose of *m*-AMSA (mg/m^2)	
60	27 (84)
100	1 (3)
120	4 (13)
Maximum dose of *m*-AMSA (mg/m^2)	
60	18 (56)
80	7 (22)
100	2 (6)
120	3 (9)
140	1 (3)
160	1 (3)
Response	
Partial	1 (3)
Stable disease	3 (9)
Progressive disease	28 (88)

m-AMSA alternating between 60 and 120 mg/m^2. The three patients who had stable disease for 2, 3, and 8 months had failed one to three prior chemotherapy regimens but were able to tolerate escalations of *m*-AMSA to 140, 100, and 160 mg/m^2, respectively. Of the 18 (56%) patients who could not be escalated over the 60-mg/m^2 starting dose, all had progressive disease.

Toxicity

Clearly, the dose-limiting toxic effect was myelosuppression (table 2). There was no significant stomatitis or hepatic, renal, or cardiac toxicity attributable to the drug.

DISCUSSION

This phase II study shows that *m*-AMSA is minimally active in previously treated patients with ovarian carcinoma. The question arises whether the activity of this drug would have been more impressive if prior treatment and performance status were more favorable (table 1). All patients but one had received doxorubicin, a drug with probable cross-resistance. Over one-half (56%) of the patients could not be escalated above the 60-mg/m^2 dose due to myelosuppression, and 12 (37%) patients received only one dose of *m*-AMSA. However, similar results were found in four other phase II studies published after 1979 in which most patients were started at 120 mg/m^2 (4–7). In these trials there were five responses (two complete responses and three partial responses) among 101 patients. In our trial, one (3%) of the 32 patients had an objective response. AMSA analogs with less myelosuppression and better distribution to abdominal sites might be more effective in this disease and perhaps should be brought to clinical trials.

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Phase II Trial of Vinzolidine, an Oral Vinca Alkaloid, in Hodgkin's Disease and Non-Hodgkin's Lymphoma[1,2,3]

Bonnie J. Takasugi, Stephen E. Jones,* and Aurelia B. Robertone[4]

Vinzolidine, a semisynthetic vinblastine derivative, was tested in an oral formulation in 21 heavily pretreated patients with lymphoma. Partial remissions were seen in four patients with Hodgkin's disease (50%) and in one patient with non-Hodgkin's lymphoma (8%). Significant side effects included neurotoxicity and dose-related myelosuppression. Vinzolidine is an active vinca alkaloid which merits further evaluation in patients with lymphoma, particularly Hodgkin's disease. [Cancer Treat Rep 68:1399–1401, 1984]

The currently available vinca alkaloids are useful in the treatment of a variety of hematologic and neoplastic disorders, but are administered iv, so that an active oral agent would be of interest. In the initial trials of vinzolidine (VZL), a semisynthetic vinblastine derivative, oral doses of 20–70 mg/m²/week were tolerated, but based on the drug's long half-life, a 14–21-day dose interval was recommended (1,2).

PATIENTS AND METHODS

Between July 1982 and May 1983, 21 patients with progressive lymphoma were entered in this study. Thirteen patients had non-Hodgkin's lymphoma (NHL), categorized by the Rappaport system as nodular lymphocytic poorly differentiated in four, diffuse lymphocytic poorly differentiated in three, diffuse histiocytic in three, nodular mixed in one, nodular histiocytic in one, and diffuse undifferentiated in one. Eight patients had Hodgkin's disease (HD), with nodular sclerosing in seven and mixed cellularity in one, according to the Lukes-Butler classification.

Patient characteristics are shown in table 1. All patients had measurable disease, creatinine < 3.0 mg/100 ml, bilirubin < 3.0 mg/100 ml, wbc count $\geq 4000/mm^3$, and platelet count $\geq 100{,}000/mm^3$, except for two patients with lower wbc counts on the basis of marrow involvement. No concurrent chemotherapy or radiotherapy was allowed and no chemotherapy was administered for 3 weeks prior to VZL.

The initial starting dose of VZL was 50 mg/m² on Day 1 every 14–21 days, depending on recovery from any toxicity. In an attempt to minimize gastrointestinal side effects, the treatment was divided into two doses taken on an empty stomach. Because of myelosuppression, the starting dose was reduced during the study, so that 12 patients started at 50 mg/m², four at 40 mg/m², and five at 30 mg/m². Subsequent dose modifications were based on the degree of toxicity. Treatment was continued until disease progression or unacceptable toxicity.

Responses, defined in a standard manner (3), were determined by serial physical examinations and, when indicated, by chest radiographs, ultrasound, or computerized tomographic studies and bone marrows. The duration of response was measured from its occurrence.

RESULTS

All patients have completed treatment with VZL and are evaluable for response. There were no complete remissions. Among the 13 patients with NHL, there was one (8%) partial remission (PR) for 10 weeks in a patient with nodular lymphocytic poorly differentiated lymphoma. In the eight patients with HD, there were four (50%) PR for 6, 9, 15, and 21 weeks.

Ninety-eight courses of VZL were administered, with a median of four courses per patient (range, one to ten). The median dose per course was 42 mg (range, 20–65), and the median total dose per patient was 180 mg (range, 40–390). Myelosuppression, determined by weekly blood

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[3]Vinzolidine was supplied in 1-, 5-, and 10-mg capsules by Eli Lilly and Co, Indianapolis, IN.

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TABLE 1.—Patient characteristics*

Characteristic	Non-Hodgkin's lymphoma	Hodgkin's disease
Total patients	13	8
Male:female	7:6	6:2
Median age in yrs (range)	56 (37-76)	29 (21-58)
Median Karnofsky performance status (range)	80 (70-100)	85 (70-100)
Prior radiotherapy	2	6
Prior chemotherapy	13	8
Median No. of regimens (range)	2 (1-5)	3 (2-4)
No. of prior vinca alkaloids		
1	7	0
2	5	5
3	1	3

*Unless otherwise specified, values = No. of patients.

cell counts, was dose-related (table 2). Three patients, two of whom had bacterial sepsis, were hospitalized with fever while granulocytopenic. Granulocyte count nadirs were generally noted 7-14 days after treatment, with recovery by Day 21. Gastrointestinal side effects were frequent, but were usually mild and did not require treatment (table 2). Two patients complained of epigastric pain, and in one, a pyloric ulcer was documented. Constipation was usually preceded by diarrhea, and two patients developed a paralytic ileus. Reversible lower extremity weakness developed in six patients (29%), who were generally older (median age, 62 years) and had received more VZL (median total dose, 265 mg/m^2) than unaffected patients. Additionally, three patients had manifested similar neurotoxicity with previous vinca alkaloid therapy. Other side effects included paresthesias in four patients, muscle cramps in six, malaise in nine, and mild alopecia in five.

DISCUSSION

This study suggests that VZL is active in lymphomas, particularly HD, with four of eight patients achieving PR despite prior treatment with two other vinca alkaloids, usually in combination with other drugs. Only one PR was obtained in 13 previously treated patients with NHL.

TABLE 2.—Relationship of toxicity to VZL dose

Toxic effect*	Dose range (mg/m^2)		
	50-65	31-49	20-30
Granulocyte count < 1000 cells/mm^3	20/41 (49)†	5/29 (17)	0/18
Grade 3	5	1	—
Grade 4	15	4	—
Platelet count < 100,000 cells/mm^3	4/44 (9)	2/29 (7)	1/21 (5)
Grade 1	1	1	1
Grade 2	3	1	—
Hemoglobin < 9.5	6/44 (14)	2/29 (7)	2/21 (10)
Grade 2	6	1	—
Grade 3	—	1	2
Nausea, vomiting	29/44 (66)	13/31 (42)	4/23 (17)
Grade 1	19	11	3
Grade 2	8	2	1
Grade 3	2	—	—
Diarrhea	24/44 (55)	7/31 (23)	3/23 (13)
Grade 1	14	6	3
Grade 2	10	1	—
Constipation	9/44 (20)	1/31 (3)	1/23 (4)
Grade 1	6	—	—
Grade 2	2	1	—
Grade 3	1	—	1

*Grades are according to Southwest Oncology Group criteria.
†Values = No. of courses with toxicity/No. of courses evaluable for toxicity (%).

The number of patients is small, because patient entry was halted after unpredictable myelosuppression was observed in concurrent studies using a different dose schedule.

With a divided dose regimen every 14–21 days, side effects appear to be predictable and acceptable. Our data support a starting dose of 30 mg/m^2 for patients with prior intensive chemotherapy or bone marrow involvement (4). Granulocytopenia, the most significant toxicity, was generally dose-related. Age and extent of prior therapy did not correlate with the degree of myelosuppression observed. The 29% incidence of significant neurotoxicity was likely influenced by previous vinca alkaloid exposure in all patients. Two patients received repeated doses of 65 mg/m^2 without severe toxicity, suggesting pharmacokinetic variability among patients that may be elucidated with testing of an iv formulation.

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Combination Chemotherapy With Cisplatin, Carmustine, Dacarbazine, and Tamoxifen in Metastatic Melanoma[1,2]

Salvatore A. Del Prete,* L. Herbert Maurer, Joseph O'Donnell, R. Jackson Forcier, and Paul LeMarbre[3]

Patients with disseminated malignant melanoma have a uniformly grave prognosis. Treatment of these patients has been unsatisfactory, with dacarbazine (DTIC), the only approved standard single agent, achieving an objective response rate between 8% and 30% (1), predominantly in patients with soft tissue or nodal involvement. Combination DTIC-based chemotherapy has not consistently made a significant change in these response rates. Here we describe an encouraging combination of cisplatin (CDDP), DTIC, carmustine (BCNU), and tamoxifen in patients with disseminated melanoma.

METHODS

Twenty consecutive patients with measurable, metastatic malignant melanoma participated in this trial. The treatment regimen consisted of: 25 mg/m^2 of CDDP and 220 mg/m^2 of DTIC iv daily for 3 days every 3 weeks, 150 mg/m^2 of BCNU iv every 6 weeks, and 10 mg of tamoxifen orally twice daily.

A complete response (CR) was defined as the disappearance of all measurable lesions and other evidence of disease; a partial response (PR) was defined as a decrease of > 50% in the sum of the products of the perpendicular dimensions of all measurable lesions for at least 8 weeks. All other patients were considered nonresponders.

RESULTS

Table 1 summarizes patient characteristics and responses to therapy.

Four patients achieved CR; all had visceral disease. Two of these patients are alive at 14 and 23 months, with disease-free intervals of 11 and 16 months, respectively; one died of BCNU pulmonary toxicity after 7 months of therapy; and one relapsed after 17 months and died 6 months later.

Seven patients achieved PR. Two of these seven patients are alive at 9 and 10 months from the onset of therapy; the other patients died of melanoma at 3, 11, 15, 15, and 15 months. Sites of response included subcutaneous tissues, skin, lymph nodes, bone, lung, and liver. Nine patients failed to respond.

Toxicity has been acceptable, with mild to moderate bone marrow depression, and moderate nausea and vomiting predominantly on Day 1 of each cycle. Renal toxicity was mild, with only two cases of slight elevation in serum creatinine. BCNU pulmonary toxicity was suspected in one patient who died of respiratory failure. In another patient, early toxicity was suspected on the basis of chest x-ray changes and pulmonary function tests. These abnormalities resolved within 2 months of the cessation of BCNU therapy.

DISCUSSION

Despite the small number of patients, our results are encouraging when compared to previous reports of chemotherapy in this disease (1-6). Prior evidence for activity of the CDDP-DTIC combination has been reported (7).

It is clear that none of the agents tested in this study, either alone or in combination with other agents, have made a significant impact on the treatment of metastatic melanona. However, when used in the doses and schedules reported here, this combination of DTIC, CDDP, BCNU, and tamoxifen appears to have greater efficacy with acceptable toxicity than the additive effects of the individual agents previously reported (1-6).

We conclude that this regimen may represent a synergistic combination worthy of further study.

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TABLE 1.—Patient characteristics and responses to therapy

			Sites of involvement													
Patient No.	Sex	Age in yrs	Regional nodes	Subcutaneous	Abdominal nodes	Bone	Lung	CNS	Liver	Small bowel	Nostril	Performance status	Response	Duration of response (mos)	Survival (mos)	Status*
1	F	73					X					1	CR	11	14	A
2	F	33					X		X			1	PR	10	10	A
3	F	45					X					1	PR	5	9	A
4	F	40	X	X			X					2	PR	14	15	D
5	F	42		X			X					2	NR‡	—	8	D
6	F	58							X			3	NR	—	2	D
7	M	65	X	X			X					2	NR	—	6	D
8	F	70	X	X			X					2	PR	9	15	D
9	M	23	X	X			X	X‡	X‡	X‡		3	PR	3	3	D
10	F	30				X						2	CR	7	7	D
11	F	59		X			X		X			2	PR	12	15	D
12	F	61	X				X				X	2	CR	17	23	D
13	F	54			X							2	NR	—	5	D
14	M	49					X					2	CR	16	23	A
15	M	40				X		X				3	NR	—	4	D
16	F	59						X	X			4	NR	—	9 days	D
17	F	51		X	X	X		X				2	NR§		3	D
18	F	31	X			X	X		X			1	NR	—	3	D
19	M	61					X		X			2	PR	5	11	D
20	F	54		X		X						1	NR	—	3	A

*A = alive; D = dead.
†NR = no response.
‡Sites not re-evaluated for various reasons.
§ Patient had received a course of DTIC in 1979, 3 years before study entry.

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Evaluation of Vindesine in Patients With Refractory Small Cell Lung Cancer[1,2]

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Vindesine is a new vinca alkaloid which has been shown to be active in patients with leukemia, lymphoma, breast cancer, and non-small cell lung cancer (1). Lack of cross-resistance between vincristine and vindesine has also been reported in patients with acute lymphoblastic leukemia (1) and breast cancer (2). We investigated the effectiveness of vindesine in patients with small cell lung cancer which had become refractory to standard combination chemotherapy.

PATIENTS AND METHODS

Twenty eligible patients with pathologically documented small cell lung cancer and a performance status of 2 or better (Eastern Cooperative Oncology Group [ECOG] criteria) were entered in the study (3). Other requirements included normal hepatic, renal, and bone marrow functions, proven failure on standard combination chemotherapy, and measurable or evaluable disease.

Vindesine was given by iv injection at 3 mg/m²/week. A 50% dose reduction was done for a wbc count between 2500 and 3999/μl or a platelet count between 75,999 and 99,999/μl. Treatment was withheld for wbc counts < 2500/μl or platelet counts < 75,000/μl. Patients were evaluated every 4 weeks with clinical and laboratory assessment of disease response using the ECOG criteria (3).

Patient characteristics are shown in table 1.

TABLE 1.—Patient characteristics and treatment results

Characteristic	No. of patients (%)
Median age in yrs (range): 57 (35-70)	
Male:female	11:9
Performance status	
0, 1	15 (75)
2	5 (25)
Extent of disease	
Limited stage	4 (20)
Extensive stage	16 (80)
Prior therapy	
Radiotherapy	6 (30)
Cyclophosphamide	20 (100)
Lomustine, methotrexate, doxorubicin, and vincristine	18 (90)
Procarbazine	14 (70)
Etoposide	5 (25)
Average No. of chemotherapy regimens: 2.3 (1-5)	
Median dose received (range): 25 mg (7-43)	
> 70% planned dose	8 (40)
< 50% planned dose	4 (20)
Median time to progression in days (range): 60 (13-92)	
Median time to death in days (range): 96 (18-208)	
Toxic effects	
Wbc count nadir (cells/μl)	
1000-1999	6 (30)
< 1000	1 (5)
Platelet count nadir (cells/μl)	
75,000-129,999	2 (10)
< 75,000	1 (5)
Anemia requiring transfusion	2 (10)
Moderate paresthesias, decreased reflex	2 (10)
Transient weakness, foot drop	1 (5)
Nausea and/or vomiting (all moderate)	3 (15)
Phlebitis	1 (5)

RESULTS

One patient had a partial response of 5 weeks' duration. This patient had previously received vincristine.

Side effects were mainly myelosuppression and neuropathy (table 1). One patient with progressive retroperitoneal metastases developed hyperuricemia (uric acid, 17.3 mg/100 ml), anuria, and acute renal failure 5 days after the first dose of vindesine. This was thought to be due to progressive disease.

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DISCUSSION

We observed only one objective partial response among 20 patients (5%). This finding is different from previous reports by Østerland et al (4) and Natale et al (5), who reported 27% (seven of 26 patients) and 21% (five of 24) objective response rates, respectively. Our results are in agreement with those of Fuks et al (6), who reported no responses in 18 patients. The accumulated response rate in these four series is 15% (13 of 88 patients). The reasons for the disparity in the response rates of the four series are not apparent, although a slightly higher dose was given to the patients in the Østerland and Natale series.

No patients in our series responded to further treatment after they were removed from the study, including six who were treated with etoposide. The median survival of 96 days is similar to that reported by Natale et al (5) (13 weeks). We conclude that vindesine has a limited therapeutic effect in patients with refractory small cell lung cancer. The relatively low response rate and short duration of time to treatment failure speaks against further phase II trials with a similar patient population and schedule. We feel that further trials of vindesine in patients with small cell lung cancer should use vindesine in the first-line combination treatment, preferably alternating with other vinca alkaloid-containing regimens or the use of a different schedule which may improve the therapeutic index.

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Phase II Evaluation of Chlorozotocin in Refractory Multiple Myeloma[1,2]

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Multiple myeloma is a chemotherapy-sensitive malignancy. Of the many complications accompanying this disease, compromised renal and bone marrow functions are the two that restrict chemotherapy treatment.

During initial trials, chlorozotocin, the chlorethyl analog of streptozocin, has been shown to be less nephrotoxic and, at equivalent doses, less myelotoxic than other nitrosoureas (1). In patients with disease refractory to standard chemotherapy (ie, carmustine, cyclophosphamide, and prednisone [BCP]; melphalan and prednisone; or prednisone, doxorubicin, azathioprine, and vincristine [PAIV]), chlorozotocin looked promising.

MATERIALS AND METHODS

All patients had a diagnosis of multiple myeloma. Using Southeastern Cancer Study Group criteria (2), all patients were categorized as good or poor risk. To begin this regimen, patients had to have the following: a measurable serum and/or urine protein abnormality, creatinine clearance > 50 ml/min, and stable wbc and platelet counts with > 2000 granulocytes and 50,000 platelets/dl.

Each course of therapy consisted of 120 mg/m² of chlorozotocin iv and 75 mg of prednisone orally, on Days 1 through 7. Therapy was repeated every 6 weeks unless toxicity or progression of the disease was noted. Progression of the disease was defined as a 25% increase in the serum protein abnormality. Patients who had a fair to excellent response were continued on therapy until their disease progressed.

RESULTS

Of the 29 patients entered in this study, 22 (75%) were evaluable for response and/or toxicity (tables 1 and 2). Seven patients were partially evaluable for toxic effects but not response, either because they died early (five patients) or because toxicity prevented further treatment (two). There were no excellent responders in the entire group. Two patients had a fair response and seven showed no progression of disease. The latter few patients had no common characteristics related to the parameters we were following. All responses were short-lived. Six patients had three or more courses of therapy before disease progression or toxicity precluded continuing the drug therapy.

TABLE 1.—Patient characteristics and response

Characteristics	No. of patients
Total entered	29
Evaluable	22
Good risk	14
Poor risk	15
Prior chemotherapy	
> 2 regimens	6
1-2 regimens	16
Cell mass	
Low	4
Intermediate	10
High	13
Unknown	2
Immunoglobulin type	
IgG	21
IgA	4
Kappa	18
Lambda	5
Light chain only	3
Response (good/poor risk)*	
Excellent	0/0
Fair	2/0
No progression	4/3

*Response criteria: *excellent*—complete disappearance of all serum myeloma protein with resolution of bone pain and restoration of all parameters to normal, *good*—a 50% reduction in these findings, and *fair*—a 25% improvement.

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TABLE 2.—Toxicity data

Toxicity	No. of good/poor risk patients with grade— 1	2	3	4
Hematologic				
Hematocrit	3/1	2/7	0/0	0/0
Wbc count	3/4	3/2	1/1	0/0
Platelet count	2/0	3/4	0/0	0/0
Renal				
BUN	2/2	0/0	0/0	0/0
Creatinine clearance	2/0	2/1	0/2	0/0
Hepatic				
SGOT	1/1	0/0	0/1	0/0
Gastrointestinal				
Nausea/vomiting	1/0	0/0	2/0	0/0

DISCUSSION

Chlorozotocin is a nitrosourea that retains its alkylating activity but lacks the carbamylation potential of the other nitrosoureas. In a phase II study, Talley et al (3) noted therapeutic activity when chlorozotocin was administered at 120 mg/m^2. They noted that this was also the greatest amount of drug that could be administered to patients previously treated with other agents, without the development of thrombocytopenia. Silver et al (4), in a solid tumor phase II trial, also noted that significant leukopenia and thrombocytopenia occurred only in patients with previous chemotherapy. Our data support these claims.

None of our patients achieved more than a 25% decrease in their myeloma protein. However, seven patients were observed to have no disease progression, ie, no increase in myeloma protein while in the study. The significance of this fact is not clear but might be relevant, since all patients, to be eligible for entry in this study, had to have progressive disease. We conclude that in the group of patients we studied, chlorozotocin was not an effective therapy.

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Phase II Trial of Amsacrine in Pancreatic Carcinoma: A Southwest Oncology Group Study[1,2]

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Amsacrine (AMSA) is an acridine derivative with limited activity in gastrointestinal malignancies (1-3). This Southwest Oncology Group study evaluated the effects of AMSA in previously treated and untreated patients with pancreatic carcinoma.

METHODS

Fifty-five patients with pancreatic carcinoma were treated in this study (SWOG 7931). All patients had a life expectancy > 6 weeks, performance status > 50%-60% (Karnofsky scale), wbc count > 4000/mm^3, platelet count > 100,000/mm^3, and bilirubin < 5 mg/dl; they had not had chemotherapy or radiation therapy within 4 weeks.

The good-risk patients received 120 mg/m^2 of AMSA every 21 days, provided there was adequate marrow recovery. Patients > 65 years of age, patients treated previously with chemotherapy and radiation therapy with suspected impaired marrow reserve, and patients with bilirubin > 2 mg/dl received 90 mg/m^2 (poor-risk). The doses of subsequent courses were adjusted according to the nadir blood counts of the previous course.

Patients were evaluated after two courses of therapy. Disease stabilization was defined as < 50% decrease in the sum of the products of the perpendicular diameters of all measured lesions.

RESULTS

Patient characteristics, treatment results, and toxic effects are summarized in table 1. No complete or partial remissions were reported in 18 evaluable patients treated with 120 mg/m^2. Six patients had stable disease after two courses of therapy. Five of 13 evaluable patients in the poor-risk group had disease stabilization, but no complete or partial remissions were noted.

TABLE 1.—Patient characteristics*

Characteristics	Good-risk	Poor-risk
Treated	26	29
Median age in yrs (range)	55 (41-64)	63 (35-82)
Prior treatment	12	21
Liver metastases	11	20
Partially evaluable	8	16
Fully evaluable	18	13
Complete/partial response	0	0
Stable	6	5
Median survival (wks)	10	7
Toxicity		
Leukopenia (< 1000 cells/mm^3)	0	3
Thrombocytopenia (< 25,000 cells/mm^3)	0	1
Nausea/vomiting uncontrollable	0	1
Congestive heart failure	0	1
Cardiac arrhythmia	0	1
Syncope	1	0
Paresthesias	0	1
Grand mal seizure	0	1

* Unless otherwise specified, values = No. of patients.

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DISCUSSION

Previous reports have demonstrated minimal activity of *m*-AMSA in pancreatic carcinoma (4,5). In this report of 31 evaluable patients with pancreatic carcinoma treated with AMSA, no complete or partial responses were noted. Eleven patients had disease stabilization. Thus, as administered in this study, AMSA had no significant activity in the treatment of pancreatic cancer.

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Phase II Trial of Etoposide in Previously Treated Small Cell Carcinoma of the Lung[1]

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Significant antitumor activity of etoposide (VP-16) in untreated small cell lung carcinoma (SCLC) has been established (1). The present study was undertaken to explore the activity of VP-16 in patients with previously treated SCLC.

METHODS

Eligibility criteria included measurable histologically documented SCLC, wbc count > 3000/μl, platelet count > 100,000/μl, bilirubin < 5.0 mg/dl, performance status of 0–2, and life expectancy > 2 months.

VP-16, 125 mg/m^2, was given iv over 1 hour on Days 1, 3, and 5 every 3 weeks. Doses were reduced for a wbc count < 3000/μl or a platelet count < 100,000/μl on the day of scheduled treatment. Standard definitions of response were used.

RESULTS

Of the 27 patients entered in the study, four were inevaluable for response (table 1). Two patients were registered but never received VP-16, one patient had no measurable disease and was thus ineligible, and one patient received no further VP-16 after hives developed immediately following the first dose.

Five patients had dose escalations and five had doses modified for myelosuppression, which was dose-limiting. Of the 23 patients evaluable for toxicity, 15 had moderate to severe (grade > 2) hematologic toxic effects. One patient had wbc count nadirs < 1000/μl and two had platelet count nadirs < 25,000/μl.

While all 23 patients had had prior chemotherapy (table 1), five had had no prior treatment with vincristine.

There were no responses to VP-16 in our study.

DISCUSSION

In a 1979 review, Radice et al (1) reported an overall

TABLE 1.—Patient characteristics

Characteristics	No. of patients
Entered	27
Evaluable	23
Inevaluable	4
Never treated	2
Ineligible	1
Allergic reaction	1
Sex	
Males	18
Females	9
Performance status	
0	4
1	7
2	16
Median age in yrs (range): 57 (33–72)	
Median No. of etoposide courses (range): 2 (1–5)	
Prior chemotherapy	
No. of combinations	
1	17*
2	7
3	3
Prior regimens†	
CAV	18*
MACC‡	3
AC	2
CAVM	2
CAV, lomustine	1
CVMF	1
Secondary and tertiary regimens†	
Phase II agents	4
Oral C	3
V	1
VC, carmustine	1
VCM	1
AC	1
Procarbazine	1
Procarbazine, M, lomustine	1

*Includes 4 inevaluable patients.

†C = cyclophosphamide; A = doxorubicin; V = vincristine; M = methotrexate; F = 5-FU.

‡MACC = methotrexate, doxorubicin, cyclophosphamide, and lomustine.

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response rate of 44% for VP-16 in SCLC. Almost one-half of these patients were previously untreated. In addition, VP-16 appears to be non-cross-resistant with vincristine in laboratory and early phase II studies.

Dose schedule appears crucial. Few responses have been observed using a weekly schedule (2,3). In contrast, using VP-16 three times per week every 4–5 weeks, a response rate > 50% was observed in patients with previously untreated SCLC, although no previously treated patients responded (4). Oral VP-16 given 3 days weekly or 5 days every 3 weeks also appears superior to treatment with weekly iv doses (5).

Although the efficacy of VP-16 is established in the treatment of patients with newly diagnosed SCLC, VP-16 should not be used as a single agent in patients failing initial therapy. Those patients should be placed on established salvage regimens when available or in phase II trials of other investigational agents.

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Phase II Study of 4-Demethoxydaunorubicin in Patients With Adenocarcinoma of the Upper Gastrointestinal Tract[1,2]

Avi Einzig, David Kelsen,* Edgar Cheng, Peter Sordillo, Virginia Raymond, and Gordon Magill[3]

4-Demethoxydaunorubicin (4-DMDR), a new anthracycline antitumor antibiotic, is a derivative of daunorubicin that lacks the methoxy group in position 4. It has shown significant activity against a variety of transplanted murine tumor lines including leukemia and solid tumors (1,2). A possible increase in therapeutic index (as compared to other anthracyclines) is suggested by animal studies which demonstrated less cardiotoxicity with equally effective doses. In a phase I study at Memorial Sloan-Kettering Cancer Center, 4-DMDR iv on an every 3 week schedule was determined to be a reasonable schedule for phase II evaluation (3). We conducted a phase II study of 4-DMDR in the treatment of patients with advanced adenocarcinomas of the upper gastrointestinal (UGI) tract.

PATIENTS AND METHODS

Twenty-six patients with histologically proven adenocarcinoma of the UGI tract were entered in this study. Patient characteristics and results are summarized in table 1. Criteria for entry in the study included measurable or evaluable disease, total wbc count > 3500/mm^3, platelet count > 100,000/mm^3, serum creatinine < 2.0 mg/dl, serum bilirubin < 1.5 mg/dl, no therapy for 4 weeks prior to entry, anticipated survival > 6 weeks, Karnofsky performance status of > 50%, prior doses of doxorubicin or daunorubicin not to exceed 200 mg/m^2, and informed consent. Thirteen (50%) patients had received no prior chemotherapy or radiation therapy.

4-DMDR was administered iv every 3 weeks. The initial dose was 12.5 mg/m^2; the dose was escalated to 15 mg/m^2 if the nadir wbc count was > 4000 cells/mm^3 and the nadir platelet count was > 100,000/mm^3. The dose was reduced to 10 mg/m^2 if the nadir wbc count was < 1000/mm^3 or the nadir platelet count was < 75,000/mm^3. Subsequent courses were held until the wbc count was > 3500/mm^3 and the platelet count was > 100,000/mm^3. Standard response criteria were employed. One course of 4-DMDR, with evaluation for response and toxicity, was considered an adequate trial. The median number of courses was two (range, one to six).

RESULTS

Twenty-five of the 26 patients received an adequate trial. Twenty-three patients experienced disease progression. One patient had a partial response lasting 3 months. One patient has stable disease (2+ months).

Toxicity was primarily hematologic and generally was observed 10–14 days after treatment. The wbc count nadir range was 300–8900 cells/mm^3, with a median of 2800; the platelet count nadir range was 41,000–561,000/mm^3, with a median of 155,000. Nausea, anorexia, vomiting, and fatigue were frequently observed. No patient developed clinical evidence of congestive heart failure.

TABLE 1.—Pretreatment characteristics and study results

	No. of patients
Entered/evaluable	26/25
Median age in yrs (range): 54.5 (24–79)	
Median Karnofsky performance status (range): 70 (60–90)	
Prior therapy	
Chemotherapy	10
Radiation therapy and chemotherapy	3
Primary site	
Esophagus	1
Gastroesophageal junction	10
Gastric	15
Complete/partial responses	0/1
Disease stabilization/minor responses	1/0
Disease progression	23

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DISCUSSION

4-DMDR appears to have minimal activity in advanced adenocarcinoma of the UGI tract (observed response rate, 4%; 95% confidence limits, 2%–19%); it appears to be less active than doxorubicin in this patient group. We plan no further studies with this agent for this disease.

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Continuous-Infusion Vinblastine for Treatment of Refractory Epithelial Carcinoma of the Ovary: A Phase II Trial[1]

John J. Kavanagh,* J. Taylor Wharton, and Felix N. Rutledge[2]

Vinblastine is a vinca alkaloid that displays a broad spectrum of activities against a number of tumor malignancies (1). The drug has demonstrated activity against epithelial ovarian cancer in human stem cell tumor assays (2). It has also been incorporated in combination chemotherapy, with positive results (3).

There is a lack of information about the effect of this drug when it is administered as a single agent in treating advanced ovarian cancer. When given as a 5-day continuous infusion, sustained blood levels of the drug may be achieved and yield clinical benefits (4,5). For these reasons, we report a phase II trial of vinblastine administered to patients with refractory epithelial ovarian cancer.

MATERIALS AND METHODS

Sixteen patients with epithelial ovarian cancer, who had failed previous chemotherapy, were treated with continuous vinblastine infusions. The starting dose was 1.0-1.4 mg/m^2/24 hours for 5 days. The lower dose was given to patients with a history of prior radiation therapy or prolonged myelosuppression with previous chemotherapy. The drug was given iv in 500 ml of 5% dextrose in water to inpatients and with chemotherapy pumps to outpatients. Most patients were outpatients. Dosage adjustments were aimed at producing granulocytopenia (500-1000 cells/mm^3) or thrombocytopenia (50,000-100,000 cells/mm^3). Standard response criteria were used. Additional patient characteristics are listed in table 1. All patients had received cisplatin previously and four patients had had only one prior chemotherapy regimen.

TABLE 1.—Patient characteristics

Characteristics	No. of patients (%)
Total evaluable	16 (100)
Median age in yrs (range): 58 (27-73)	
Performance status (Zubrod scale)	
1	10 (62)
2	3 (19)
3	3 (19)
Prior chemotherapy: cisplatin combinations	16 (100)
Median No. of regimens: 2	
Vinblastine dose (mg/m^2)	
1.6-2.0	6
1.4	6
1.2	8
1.0	2
Granulocyte count nadir (cells/mm^3)	
Median No. (range): 1400 (300-5000)	
Courses ≤ 1000: 45%	
Courses ≤ 500: 21%	

RESULTS

Sixteen patients received 29 courses of therapy. The median number of courses given was two. All patients were considered to be evaluable. There were no complete or partial responders.

Two patients were stable for 2 and 5 months. Five patients required hospitalization for complications related to neutropenia. One patient developed mild neuropathy. Three patients had constipation and three developed self-resolving ileuses. One patient developed mild transient thrombocytopenia after one course.

The lack of response and the gastrointestinal side effects indicate that further study of the treatment of refractory epithelial ovarian cancer with vinblastine at these doses and method of administration is unwarranted.

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Response of a Hepatocellular Carcinoma to the Cisplatin-Vinblastine-Bleomycin Drug Regimen

We recently observed a case of hepatocellular carcinoma that responded to the cisplatin-vinblastine-bleomycin (PVB) drug regimen. The case is being presented because we feel that this combination of agents warrants further study in primary carcinomas of the liver.

CASE REPORT

A 62-year-old white male patient presented in October 1982 with right upper quadrant tenderness and melena. The past history was significant for heavy alcohol use and a colectomy with ileostomy in 1958 for ulcerative colitis. Physical examination revealed mild tenderness to deep palpation of the right upper quadrant, but no abdominal masses or organomegaly. An upper abdominal sonogram revealed a 4-cm mass in the region of the celiac axis consistent with adenopathy, and in addition, showed evidence of portal vein thrombosis and a questionable focal lesion in the left lobe of the liver. Liver spleen scan showed multiple hepatic defects. Angiography revealed celiac stenosis with slow filling of the hepatic artery, but no hypervascularity suggestive of hepatoma. The serum alpha-fetoprotein was elevated to 18,850 ng/ml on October 6, 1982, and to 23,300 ng/ml on October 15, 1982. HBsAg was negative. Peritoneoscopy with biopsy was planned, but in January 1983, the patient developed disorientation and left-sided weakness. A computerized axial tomographic scan of the brain showed enhancing cystic lesions bilaterally with a right to left midline shift. A craniotomy was performed and biopsy showed an undifferentiated carcinoma with compact sheets of monomorphic polygonal cells and marked mitotic activity (14 mitoses per high power field). Few neoplastic cells exhibited a positive reaction for alpha-fetoprotein and beta-human chorionic gonadotropin (HCG) by the PAP technique (1). A repeat serum alpha-fetoprotein on January 7, 1983 was 30,730 ng/ml. The serum beta-HCG was within normal limits. A testicular sonogram was normal.

The patient received 3000 rads of whole-brain irradiation and was started on chemotherapy for a possible extra gonadal germ cell tumor. The chemotherapy consisted of 100 mg/m^2 of cisplatin on Day 1, 0.15 mg/kg of vinblastine on Days 1 and 2, and 30 U of bleomycin iv weekly, with cycles repeating every 21 days. The patient did extremely well, with complete resolution of his left-sided neurologic deficits. Alpha-fetoprotein dropped progressively with each course of therapy (20,345 ng/ml on February 11, 1983, 12,920 ng/ml on March 13, 1983, and 3940 ng/ml on March 17, 1983.

Nine days after initiation of the third cycle of chemotherapy, the patient was admitted with nausea, poor oral intake, dehydration, hypotension, fever, melena, renal failure, and severe pancytopenia. The clinical impression was that of chemotherapy-induced sepsis and bleeding. The patient died on March 20, 1983, despite support with fluids, antibiotics, and blood and platelet transfusions.

At autopsy, the immediate cause of death was found to be massive pulmonary embolism. In addition, there was evidence of a terminal *Candida* sepsis with microabscesses in the right kidney and the myocardium.

Both lobes of the liver contained multiple tumor nodules measuring up to 3 cm in diameter. There was marked tumor invasion with obstruction of the portal vein and its branches. Metastatic carcinoma was found in portal and peripancreatic lymph nodes along the celiac axis. In addition, multiple metastatic lesions were present in the brain, involving the frontal, parietal, and occipital lobes and the cerebellum. Most of these lesions were of small size, measuring < 2 cm in diameter. All other organs were free of tumor. Histologic sections of the hepatic and metastatic lesions revealed extensive tumor necrosis with only a small rim of viable-appearing tumor tissue present along the periphery of the necrotic areas. The residual tumor showed marked cellular pleomorphism and only few mitoses (one mitosis per high power field). In a number of brain sections, hepatocellular carcinoma was confirmed by the presence of intracytoplasmic and intercellular bile droplets that were positive for bilirubin by Hall's technique (2). Retrospective review of the original brain biopsy also revealed a few small intracytoplasmic bile granules.

The liver parenchyma showed marked portal fibrosis but no cirrhosis.

DISCUSSION

Treatment of this patient with the PVB drug regimen, combined with radiation therapy to the brain (3000 rads), produced a significant decrease in the serum level of alpha-fetoprotein. While both treatment modalities must have contributed to the decline of the alpha-fetoprotein level, it is believed that chemotherapy had a significant effect, since no radiation therapy was applied to the up-

per abdominal area, the site of the primary tumor and regional lymph node metastases. This decline continued for 2 months following completion of radiation therapy coincident with further chemotherapy. The therapy effect reflected also on the morphology of the tumor, which in the biopsy taken prior to treatment had the appearance of an anaplastic, rapidly growing neoplasm with an extremely high mitotic rate. In the sections taken at autopsy, the tumor was mostly necrotic with the residual viable-appearing cells showing a low mitotic rate.

The question is raised whether hepatocellular carcinomas, particularly those with markers present also in germ tumors (alpha-fetoprotein, beta-HCG), may respond to the same drug combination that is effective in germ cell tumors. To our knowledge, the PVB drug regimen has not previously been studied in hepatocellular carcinoma. Intra-arterial cisplatin infusion has been investigated in primary liver carcinoma with a 20% response rate obtained in a phase II trial (3).

In view of the results presented, further trials with the PVB drug regimen in cases of hepatocellular carcinoma should be carried out.

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Hypercalcemic Flare With Megestrol Acetate

We report a case of hypercalcemia precipitated by megestrol acetate in a woman with breast cancer and extensive bony metastases. Exacerbation of bone pain and soft tissue metastases has been reported (1), but we are not aware of any reports of hypercalcemia with this drug. On January 6, 1984, our patient presented with pain in her left thigh. She had received cyclophosphamide, 5-FU, and prednisone (CFP) since October 1981. Apart from the thigh discomfort, she was asymptomatic and worked full-time as a clerk. Physical examination was unremarkable. Roentgenograms showed progressive lytic disease of the opposite femur and a new rib fracture. The patient received prophylactic radiotherapy to the right femur. She became asymptomatic and was seen again on January 23, at which time she had a normal serum calcium of 10.0 mg/dl and creatinine of 1.0 mg/dl. Megestrol acetate, 40 mg orally four times daily, was begun. Other medications included Dyazide (one capsule daily). Over the next 4 days, the patient developed severe diffuse bone pain, polydipsia, nausea, vomiting, and constipation. She stopped all medications after ingesting a total of only 16 megestrol acetate tablets. On January 30, serum calcium was 22.6 mg/dl and creatinine was 1.4 mg/dl. Several new rib fractures and compression fracture of T-12 had developed.

The patient was hospitalized and received furosemide (40 mg/day), saline hydration (250 ml/hour), and prednisone (80 mg/day) for the first week with correction of calcium to 12.6 mg/dl. Mithramycin (1000 μg iv) was given on February 6. Aminoglutethimide (AG) (125 mg four times a day) and hydrocortisone (HC) (100 mg daily) were also begun then. Megestrol acetate was not restarted.

The patient was re-hospitalized on February 20 for treatment of a pathologic humeral fracture. She had developed several new rib fractures, pulmonary infiltrates, and a pleural effusion. Her calcium was 9.4 mg/dl on a regimen of oral hydration (2000 ml/day), furosemide (40 mg/day), and AG (1 g/day) + HC (40 mg/day). Apart from AG and HC, she received no other antitumor or antihypercalcemia treatment from February 20 to March 13 (assuming the effects of mithramycin last 2 weeks).

On March 13, the patient's pulmonary disease had progressed further and she now had mild elevation of calcium to 11.1 mg/dl. Doxorubicin was begun and AG was stopped. She has since had resolution of her pulmonary infiltrates and pleural effusion, improvement in bone

pain and performance status, and no further hypercalcemia.

We did not feel we could ethically rechallenge our patient with megestrol acetate to establish causality. We recognize, as Tormey et al (2) have pointed out, that a tumor "flare" may often be nothing more than a reflection of pre-existing rapid tumor growth. Our patient did have disease progression prior to megestrol acetate. However, this case does fit the definition of "flare" as used by Greenwald (1) as an instance of markedly increased bone pain related to the initiation of hormonal therapy.

Our patient had minimal bone pain prior to megestrol acetate, and moderate bone pain and minimal hypercalcemia on ineffective hormonal therapy after the cessation of megestrol acetate, all in the setting of progressive pulmonary disease. The rapid onset of her severe bone pain and hypercalcemia persuades us that the megestrol acetate played an integral role in causation.

This case suggests that patients with breast cancer and bony metastases are not entirely free of the risk of hypercalcemic flare with the initiation of megestrol acetate. This complication must be infrequent. In a review of 421 patients with advanced breast cancer, there is no mention of exacerbation of bone pain or hypercalcemia as adverse side effects (3). This stands in contrast to six of 45 patients with a flare when starting tamoxifen (4). Hypercalcemia has been reported following the administration of medroxyprogesterone acetate, another progestin (5). A similarity between these cases and our patient should be noted. In three of the four cases reported by Kaufman et al (5), hypercalcemia also complicated estrogen therapy given either before or after progestin therapy. In our patient, tamoxifen was stopped in the past because of acutely increased bone pain, a new compression fracture, and mild hypercalcemia. Whether levels of hormone receptor are pertinent is unknown. Our patient's tumor was estrogen receptor-positive (84 fmols/mg of cytosol protein). Progesterone receptor analysis was not done. We suggest that a past history of flare with additive hormonal therapy may identify patients at risk for similar problems when beginning megestrol acetate.

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Tamoxifen and Conjugated Estrogens (Premarin) Followed by Sequenced Methotrexate and 5-FU in Refractory Advanced Breast Cancer

Following the recent reports by Allegra et al (1,2) of high response rates to sequential tamoxifen and conjugated estrogens (Premarin) combined with methotrexate and 5-FU, the National Cancer Institute of Canada Clinical Trials Group conducted a pilot study of the regimen in a heterogeneous group of patients with advanced breast cancer.

Between December 1981 and August 1982, 35 eligible patients were entered in the study and 30 were ultimately evaluable. Both premenopausal and postmenopausal patients were eligible regardless of estrogen receptor status, and all had a Karnofsky performance status of 40 or greater. The treatment given was identical to that described by Allegra et al, with the exception that cycles were repeated every 21 rather than every 18 days. Tamoxifen (10 mg orally twice a day) was given on Days 1-10, Premarin (0.625 mg orally twice a day) was given on Days 11-14, and methotrexate (200 mg/m^2 iv) was given on Day 14 followed 1 hour later by 5-FU (600 mg/m^2 iv). Folinic acid (10 mg/m^2 orally every 6 hours × six doses) was given 24 hours after the methotrexate. Doses of methotrexate and 5-FU only were modified for myelosuppression or gastrointestinal toxicity.

The patient characteristics are shown in table 1. Of the 30 patients, this treatment represented the first systemic therapy of advanced disease in nine, and the first exposure to chemotherapy in 13. Sixteen patients had one site of metastatic disease, 13 had two sites, and one had three sites.

The results of the combination chemo-hormonal therapy were disappointing. Only three of the 30 patients responded (one complete and two partial responses). Of the

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total evaluable	30
Median age in yrs (range): 55 (30–74)	
Median performance status (range): 90 (40–100)	
Menopausal status	
Premenopausal	11
Postmenopausal	19
Estrogen receptor status	
Positive	11
Negative	9
Unknown	10
Mean disease-free interval in mos (range): 23 (0–108)	
Prior treatment of metastatic disease	
Hormonal therapy	16 (14 tamoxifen)
No hormonal therapy	14
≥ 1 chemotherapy combinations	17
No prior chemotherapy	13
Neither hormones nor chemotherapy	9

13 patients for whom this treatment was the first chemotherapy for their metastatic disease, there was only one response (partial), and this individual was one of the nine who had also had no hormonal therapy. Toxicity was mild overall. Eight patients had moderate to severe nausea and vomiting, and one with borderline renal function exhibited severe reversible myelosuppression. The majority of treatment cycles (128 of 146) were given in full doses.

The results of this pilot study did not duplicate the impressive complete and overall response rate of Allegra et al. The explanation of this discrepancy is likely due, in part, to the differences in the population treated. The majority of Allegra's patients were previously untreated, and about one-half had nonvisceral dominant disease. One-third of our patients were untreated and only five of 30 had soft tissue disease. Nevertheless, it remains uncertain if this chemo-hormonal therapy is as exciting as initially reported when used in less well-selected cases of advanced breast cancer.

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Prognostic Value of Changes In Tumor Marker Concentrations During Treatment of Patients With Testicular Cancer

Since the introduction of cisplatin in combination with other drugs, especially bleomycin and vinblastine, in the treatment of germ cell tumors, the results of treatment have greatly improved. However, some patients with nonseminomatous tumors still have a poor prognosis. The prognosis is largely correlated to the tumor burden and the presence of high levels of tumor markers, ie, alpha-fetoprotein (AFP) and/or human chorionic gonadotropin (HCG) in the serum. It is now apparently possible to increase the therapeutic outcome of the treatment either by increasing the dose of cisplatin or by adding etoposide to the treatment schedule, or both (1). As such therapy significantly adds to treatment-related morbidity, a need for an early indication of the final outcome of conventional chemotherapy has emerged. Calculation of half-lives of the rate of decline of serum concentration

TABLE 1.—Serum concentration of HCG at Day 22 and of AFP at Day 43, compared with Day 1 of treatment

	No. of patients with—						
	HCG ratios of—			AFP ratios of—			Combined prediction of outcome
	HCG	< 0.005	> 0.005	AFP	< 0.0025	> 0.0025	
Patients with continuous NED*	4	3	1†	8	8	0	7/8
Patients with relapses	12	4	8	7	4	3	10/12

*NED = no evidence of disease.
†At Day 43 without HCG.

of tumor markers *during* therapy has been suggested as a useful prognostic parameter (2,3). To test the usefulness of such an approach, we retrospectively analyzed the data from 20 patients with disseminated nonseminomatous germ cell tumors and elevated tumor markers. All patients were initially treated with a combination of cisplatin, vinblastine, and bleomycin (PVB) according to Einhorn. Six treatment series with 3-week intervals were scheduled for each patient. All patients were followed with serial measurements of serum tumor markers. Eighteen months after initiation of treatment, eight patients were still in complete remission, while 12 had experienced an initial response followed by a relapse; six of these 12 were salvaged by further treatment, including etoposide. The concentrations of HCG and AFP before treatment were compared with the values at Day 22 for HCG, ie, at the start of the second PVB treatment cycle, and at Day 43 for AFP, ie, at the start of the third PVB treatment cycle. We tested the hypothesis that if the ratio of HCG concentration at Day 22 divided by corresponding value of Day 1 was > 0.005, a relapse could be anticipated. The ratio cutoff level of 0.005 was selected to make direct comparison with the observations of Picozzi et al (3) possible. Furthermore, the value of measuring AFP concentrations was tested. Due to a longer half-life for AFP, the decline is more slow; thus, we chose to use the AFP value, measured at Day 43. As the test level, a ratio of 0.0025 was selected as the value which will exist if all production of the protein is stopped and a half-life of 5 days is assumed. The results of the analysis are shown in table 1. Seven of eight patients in whom increased levels of tumor markers had declined as hopefully expected are continuously without evidence of disease. The patient in whom the HCG ratio at Day 22 was > 0.005 had a very high pretreatment serum concentration of HCG (500,000). However, at Day 43, HCG was not detectable in serum, indicating that at Day 43, eight of eight patients could correctly be attributed a good prognosis.

Among the 12 relapses, eight could be predicted at Day 22 based on HCG level calculations, while a further two patients could be predicted at Day 43 based on AFP level calculations. These results are of clinical relevance, since at Day 43 no significant difference was observed in the clinical status between patients with good and bad prognosis, respectively. Thus, tumor marker ratios are helpful in evaluation of the final outcome of chemotherapy.

Picozzi et al (3) found no use of AFP results in the prognostication. In our series, all three patients with AFP ratios > 0.0025 at Day 43 relapsed. Two of these relapses would not have been predicted if evaluation of AFP concentration values was omitted. This means that by this simple analysis, an overall predictive value of 90% should be possible.

We suggest that marker-positive patients who do not experience a decrease in the serum tumor marker concentration as indicated above should be submitted to more intensive treatment immediately, even though a response in clinico-radiological sense might prevail.

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Impact of Questionable Cancer Treatments on Oncology Practice Today

Los Angeles, California, February 6, 1985

This meeting will be held in the Biltmore Hotel, under the sponsorship of the Hospital of the Good Samaritan. It is acceptable for up to 6 hours of Category 1 credit towards CMA and AMA certificates in Continuing Medical Education; the fee (luncheon included) is $85 for practicing physicians and $45 for allied health professionals, interns, residents, fellows, and retired physicians.

For further information, contact: Dolores Gay, The Hospital of the Good Samaritan, 616 S Witmer St, Los Angeles, CA 90017 (telephone: 213-977-2352).

EORTC Symposium on Interferon in Cancer Therapy

Brussels, Belgium, February 28, 1985

This symposium is being organized under the auspices of the European Organization for Research on Treatment of Cancer. The program will include invited presentations, as well as free communications selected on the basis of submitted abstracts. Abstracts must be received at the EORTC Data Center by *December 15, 1984*. Since seating is limited, preregistration is recommended. The registration fee is US $70.

For further information and abstract forms, contact: Ms. D. Eeckhoudt, EORTC Data Center, Boulevard de Waterloo 125, 1000 Brussels, Belgium [telephone: (2) 539.28.05; telex: 22773].

EORTC Symposium on Continuous Infusion Chemotherapy

Brussels, Belgium, March 1, 1985

The European Organization for Research on Treatment of Cancer is organizing this symposium, which will include invited presentations, as well as free communications selected on the basis of submitted abstracts. Abstracts must be received at the EORTC Data Center by *December 15, 1984*. Since seating is limited, preregistration is recommended. The registration fee is US $70.

For further information and abstract forms, contact: Ms. D. Eeckhoudt, EORTC Data Center, Boulevard de Waterloo 125, 1000 Brussels, Belgium [telephone: (2) 539.28.05; telex: 22773].

Sixty-seventh Annual Meeting of the American Radium Society

Acapulco, Mexico, April 27–May 1, 1985

For information contact: Ms. Suzanne Bohn, Executive Secretary, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

Third European Conference on Clinical Oncology and Cancer Nursing

Stockholm, Sweden, June 16–20, 1985

The scientific program of this conference will emphasize a multidisciplinary approach to the study and treatment of cancer and caring for patients. The program will include posters and free papers, workshops, plenary and teaching lectures, symposia, satellite symposia, and "Ask the Specialist" sessions. All sessions will be held at the Stockholm International Fairs, "Mässan."

The deadline for registration and receipt of abstracts is *January 10, 1985*. The registration fee is SEK 2100 (SEK 1100 for registered nurses, radiologic technicians, and medical secretaries). There will be an additional charge for late registration.

For further information, contact: Mrs. Ira Thilén, Stockholm Convention Bureau, Jakobs Torg 3, S-111 52 Stockholm, Sweden (telephone: 08/23-09-90).

Conference on Chemical Modifiers of Cancer Treatment

Clearwater, Florida, October 20–24, 1985

The fifth in a series of international conferences on radiation sensitization, radioprotectors, and chemical modifiers of cancer treatment will be held at the Sheraton-Sand Key in Clearwater. The subject of this conference is the use of chemical agents (primarily radiosensitizers, radioprotectors, repair inhibitors, and agents which improve tumor oxygenation) to modify the response of tumor and/or normal tissues to the cytotoxic effects of radiation or chemotherapeutic drugs. Basic scientists in chemistry and biology and clinicians in radiotherapy and chemotherapy will exchange information and ideas on current research, future efforts, and design and implementation of new clinical trials.

The program will consist of eight sessions. It is hoped that financial assistance can be offered to a number of young investigators. The deadline for submission of abstracts, poster presentations, and requests for travel assistance is *June 15, 1985*.

For further information, contact: Ms. Suzanne Bohn, American College of Radiology, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3150).

I Congreso Iberoamericano de Oncologia

Madrid, Spain, November 3–6, 1985

This Congress has been organized to evaluate current status, approaches, and research plans in clinical and cooperative studies done by Spanish- and Portuguese-speaking oncologists. The program will include invited presentations and free communications selected on the basis of submitted abstracts.

For further information, contact: Dr. H. Cortes Funes, General Secretary, Asociacion Española Contra el Cancer, calle Amador de los Rios 5, Madrid 4, Spain.

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NEW NCI MONOGRAPHS

Management Operations of the National Cancer Institute That Influence the Governance of Science

Monograph No. 64, May 1984

Edited by Dr. Vincent T. DeVita, Director, National Cancer Institute, and Louis M. Carrese, Associate Director for Program Planning and Analysis, National Cancer Institute

By combining three new articles with the various directives on the contract and grant processes in one volume, the editors hope to dispel the misunderstandings that have arisen from incorrect or insufficient information. This monograph should serve as a useful reference document for scientists in various disciplines who wish to know more about the administrative aspects of the National Cancer Institute.
Price: $9.00 (U.S.), $11.25 (elsewhere). GPO Stock #017-042-00175-7.

Use of Small Fish Species in Carcinogenicity Testing

Monograph No. 65, May 1984

Edited by Dr. Karen L. Hoover, Laboratory of Comparative Carcinogenesis, National Cancer Institute

Included in these proceedings of a conference are the formal papers presented on the value of fish in field and laboratory studies and on the environmental surveys among larger feral fish in water pollution investigations, a panel discussion, and a consensus report on recommendations on present technical problems and future directions of research in this field.
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